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# Experiments on Multiple Factors Affecting Pinaceae Invasions on Isla Victoria, Nahuel Huapi National Park, Argentina

Martin Andres Nuñez
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To the Graduate Council:

I am submitting herewith a dissertation written by Martin Andres Nuñez entitled "Experiments on Multiple Factors Affecting Pinaceae Invasions on Isla Victoria, Nahuel Huapi National Park, Argentina." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

Daniel Simberloff, Major Professor

We have read this dissertation and recommend its acceptance:

Aimee Classen, Karen Hughes, Nathan Sanders

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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# Experiments on multiple factors affecting Pinaceae invasions on Isla Victoria, Nahuel Huapi National Park, Argentina.

A Dissertation presented for the Doctor of Philosophy Degree

The University of Tennessee, Knoxville

Martin Andres Nuñez

May 2008

# DEDICATION

This dissertation is dedicated to my wife Romina, my parents Olga and Mario, and my aunt Lucy for their unconditional support.

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# ABSTRACT

Invasive species are one of the greatest threats to global biodiversity. Therefore, to conserve biodiversity and manage exotic species effectively, it is crucial to determine the factors that regulate biological invasions. Historically, the study of invasions has focused on the dynamics and characteristics of successful invasive species. While studying successful invaders has enhanced our understanding of biological invasions, studying the failures can also illuminate the factors that limit invasions. To further understand the factors limiting the spread of exotic species, I studied invasion dynamics of several species in the family Pinaceae on Isla Victoria, Argentina. Approximately 80 years ago, thousands of trees of at least 135 non-native tree species were planted on Isla Victoria, many of them in the Pinaceae, but few species have escaped the plantation. I tested whether herbivory by exotic deer, seed predation or mycorrhizal facilitation might limit the spread of pine species from plantations. To test whether preferential herbivory by deer on non-native species plays a role, I conducted a cafeteria experiment. Deer browsed more intensively on native species than on exotics, suggesting that deer could potentially facilitate invasion by exotic Pinaceae. To test if seed predation limits exotic conifer establishment, I studied seed predation using field experiments. Seeds of exotics were preferred over seeds of native species, and predation was more intense in areas far from plantations than near plantations, reducing the chances of exotic seed establishment. To test the role of ectomycorrhizal symbiosis on invasion I conducted a series of experiments, in which I found that the lack of proper ectomycorrhizal fungi could limit invasion. Seedling establishment and growth rates were higher near inoculum sources (plantations) than far from such sources. Ectomycorrhizal colonization rates where higher

near plantations than far from them, showing that mycorrhizal interactions could be important for understanding plant invasion. Together these studies suggest that pine invasion in Isla Victoria can be controlled by a suite of, to date, underappreciated factors.

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## Introduction

Biological invasions generate economic (Pimentel *et al.* 2005), ecological (Mack *et al.* 2000, D'Antonio *et al.* 2001), and cultural (Nuñez and Simberloff 2005) damage. Despite the importance of invasions, why some species become invasive and others do not is often mysterious (Enserink 1999). Research on invasive species has focused mostly on invasive populations. This focus on successful invaders is important in understanding their overall importance as a threat to global biodiversity and why an invasive species is invasive. Consequently, the study of why some populations of exotic species are not invading attracts little research attention, probably because it is seen as more urgent or rewarding to understand species that are already invading. While studying successful invaders has clearly enhanced our understanding of biological invasions, significant progress can also be achieved by studying non-invasive populations of exotic species that are invasive elsewhere, since such study provides the key to knowing which factors are halting an invasion rather than information on the factors promoting them.

Isla Victoria, in Nahuel Huapi National Park, Argentina (Fig. I-1), offers an excellent system for studying failed invasions, because ca. 80 year ago 135 species of exotic trees were planted but few are colonizing outside the original plantations (Figures appear in the appendix). In this chapter, I will describe the island, the history of its exotic tree plantations, the observed invasion patterns of conifers, and factors proposed to explain

conifer invasion (or lack of it) occurring in the area. Subsequent chapters will address in detail the hypotheses proposed to explain the invasion pattern.

## Isla Victoria and its plantations

Isla Victoria, in Nahuel Huapi National Park, Argentina (Fig. I-1), is 20 km long and 4 km across at its widest point. In 1902 the island began to suffer major damage from logging, cattle ranching, and fires, which affected as much as 50-60% of its area. With the establishment of the Nahuel Huapi National Park in 1934, such activities substantially decreased, although a plant nursery functioned into the 1960s and cattle were not removed until ca. 1960 (Basti 1988). Today most of the island is covered either by primary or substantial secondary forest dominated by coihue (*Nothofagus dombeyi*) and ciprés (*Austrocedrus chilensis*) trees with dense understory vegetation. Shrubland communities are also present on the island, and they are dominated by ñire (*Nothofagus antarctica*) and arrayan (*Luma apiculata*) (Fig. I-1). Several roads, trails, buildings, and abandoned pastures remain on the island, with the greatest number of buildings and roads located in the Puerto Anchorena area (Fig. I-1).

Old World deer were introduced to the region of Nahuel Huapi National Park between 1917 and 1922, and both red deer *(Cervus elaphu*s) and fallow deer *(Dama dam*a) have long been on Isla Victoria (Relva and Caldiz 1998). Native deer pudu (*Pudu pudu*) and huemul (*Hippocamelus bisulcus*) have become very rare (pudu, believed to dwell only in the northern tip of the island) or extinct (huemul) on the island, but the exotic red (*Cervus*

*elaphus*) and fallow deer (*Dama dama*) are abundant. Anziano (1962) estimated their population density at 40 $km^{-2}$, and A. Relva in 2004 estimated their density at 26 $km^{-2}$ (pers. comm.); well-trodden deer trails are found throughout the island. This area is also populated by two native granivorous, nocturnal rodents, *Oligoryzomys longicaudatus* and *Abrothrix olivaceus* (both species in the Muridae: Sigmodontidae) (Cristtie *et al.* 1984). There is no record of exotic rodents on the island, but their presence is possible. The island is also populated by birds that eat seeds, such as the exotic bird *Phasianus colchicus* and the natives *Phygilus patagonicus* and *Zonotrichia capensis.* Wild boar (*Sus scrofa*) invaded the island in 1999 and currently has a substantial population, with noticeable effects throughout the island.

In 1925, the Argentine government established a nursery to grow forestry and fruit trees that might be suitable in the region. At least a few species had already been introduced beginning in 1910 (Koutché 1942, APNA 1988). The last plantings took place in 1939 (Basti 1988), although the nursery functioned until the 1960s (Basti 1988). Through 1939, at least 73 conifers – including 60% of the world's known invasive conifer species – and 62 broad leaved species were planted on the island. These included species from every continent but Antarctica. Among them were 21 species of the genus *Pinu*s (Table I-1), including 9 of the 12 species that Rejmánek and Richardson (1996) cite as having invasive qualities, and 43 species of Pinaceae, including 17 of the 28 species of Pinaceae Richardson and Rejmánek (2004) found to be invasive in different areas of the world. For most species it is impossible to determine the exact number of individuals planted and their stages, but the numbers were often very large. For instance, in 1926–

1927, for 17 conifer species, a total of 8,000 plants aged 3–5 years were planted. In 1937–1938, at Puerto Pampa (Fig. I-1), for just 6 introduced species, 101,000 plants aged 1–3 years were planted. For Scots pine *(Pinus sylvestris*), 42,645 individuals were planted. There has been no recent survey of which species remain on Isla Victoria, but at least 50 are apparent to casual observation (Simberloff *et al.* 2002), occupying an extensive area (Fig. I-1).

**Pattern of conifer invasion on Isla Victoria**

In 2000, Simberloff, Relva and Nuñez conducted a survey of exotic trees outside plantations on Isla Victoria (Simberloff *et al.* 2002). They ran a series of transects 10 m wide and 100 m apart across the width of the island outside plantations from the Puerto Pampa plantations southward through the Puerto Anchorena plantations, and they recorded every individual of exotic tree species, from seedlings to adults. They found that few species were invading and that exotics were almost absent from any region over 1000 m from the plantations (Fig. I-1, Simberloff *et al.* 2002). These results were surprising given the number of species known to be invasive elsewhere planted on Isla Victoria and the characteristics of the island (including the fact that the forests are almost wholly dominated by just two native species: *Nothofagus dombeyi* and *Austrocedrus chilensis*), raising questions about the factors explaining this invasion pattern.

## Factors and mechanisms affecting Pinaceae invasion

Many factors and mechanisms have been proposed for the success and failure of invasion among Pinaceae. These include species-specific factors such as growth rates (Grotkopp *et al.* 2002), length of juvenile period (Rejmánek and Richardson 1996), dispersal abilities (Rejmánek and Richardson 1996), and seed germination rates (Rejmánek and Richardson 1996). Other proposed mechanisms and factors related to the interaction between the species and its habitat include damage by natural enemies (Simberloff *et al.* 2002), facilitation by ectomycorrhizal fungi (Richardson *et al.* 2000), disturbance (Richardson *et al.* 1994, Simberloff *et al.* 2003), seed predation (Caccia and Ballare 1998), biotic inertia (Von Holle *et al.* 2003), secondary dispersal (Richardson and Rejmánek 2004), competition with native flora (Adamowski 2004), allelopathy (Simberloff *et al.* 2002), soil characteristics (Rouget *et al.* 2001, Simberloff *et al.* 2002), and climatic conditions (Richardson and Bond 1991). However, empirical tests of many of these factors and mechanisms remain rare for the Pinaceae.

## Hypotheses tested and not tested on this dissertation

I tested a subset of the aforementioned proposed hypotheses, because literature reviews of factors controlling pine establishment and survival in native and exotic ecosystems, the observed patterns of invasion on Isla Victoria, and preliminary results on the importance of different mechanisms on the island all suggest that herbivory by exotic deer (chapter II), post-dispersal seed predation (chapter III), and mycorrhizal facilitation (chapter IV) are most likely to govern Pinaceae invasion in this area. I did not test effects

of other factors known to control invasive species such as climatic factors, dispersal abilities, competition with native species, anthropogenic disturbance, or species-specific traits because:

1) Climatic factors are not likely important in controlling pine invasion on Isla Victoria, given that some species originate from regions with similar climate to that of Isla Victoria and invade similar regions. *Pinus ponderosa, P. sylvestris* and *P. contorta*, species planted on the island that have not become invasive, have large distributions in their native ranges and survive under a wide range of environmental conditions (Burns and Honkala 1990). These species are successful invaders in New Zealand (Table I-1), an island with regions with a comparable climate to that of our study area and that encompasses its latitude.

2) Given the seed dispersal abilities of Pinaceae and the time – in some cases more than 80 years – that the species planted on the island have had to disperse their seeds, lack of seed dispersal is not a likely factor, at least within a few hundred meters of the plantations. Studies of seed dispersal in pines show that even when most of the seeds fall close to the parent tree, a number of seeds can be dispersed long distances, hundreds or thousands of meters (Greene and Johnson 1989, Nathan *et al.* 2000), and long-distance dispersal has been shown to be extremely important for pine invasion (Higgins and Richardson 1999)

Pine colonization in North America after quaternary glaciations offers a good example; the average rates of northward migration were 400 m $y^{-1}$ for *Pinus banksiana* in eastern North America and 670 m $y^{-1}$ for *P. contorta* in costal Canada and Alaska (Macdonald *et al.* 1998). Both these species were planted on Isla Victoria without becoming invasive, although they invade elsewhere (Table I-1). Another factor that makes seed dispersal failure unlikely is that winds in the area are severe, come from different directions, and sometimes exceed 100 km $h^{-1}$ (APNA 1988).

3) The presence of abandoned farms in different areas of the island, which lack native vegetation and yet are not noticeably invaded by Pinaceae, suggests that competition with native flora is not a likely key factor. These farms lack trees and have sparse ground cover, which minimizes the chances of competitive exclusion by native flora.

4) The absence of invasion in the old agricultural farms mentioned in (3) and in other disturbed areas, such as roadsides, suggests that lack of human disturbance is not limiting invasion by some of these species.

5) I emphasize that it is not biology generally unsuitable for invasion that has prevented some of these species from spreading. Some species that are invasive elsewhere and are predicted to be invasive by virtue of growth rate, seed size and duration of the juvenile period (for example *Pinus contorta* and *P. silvestris*) are not invading this area.

There have been some studies on the proposed factors to explain invasion. For example, Caccia and Ballare (1998) found that seed predation can control regeneration of *Pseudotsuga menziesii* in northern Patagonia. Davis *et al.* (1996) found that herbivory by rabbits and lack of colonization by ectomycorrhizae may cause failure of sowing trials for *Pseudotsuga menziesii*, *Pinus radiata* and *P. nigra*. Lamb (1979) found that the absence of ectomycorrhizal fungi was one cause of the low success rates for direct sowing trials with *Pinus radiata* and *P. elliotti* in Australia. The goal of most of these projects was to find low-cost afforestation techniques, a research focus that is problematic for extrapolation to natural systems. For example, Davis *et al.* (1996) did not control for inadvertent seed inoculation with fungal spores during handling, which can be overcome by sterilizing seed surfaces. Caccia and Ballares (1998) experimented with an abnormally high number of seeds (30 seeds in a 6.3 $cm^2$ surface), which could affect seed predator behavior. For this dissertation, I conducted a series of studies designed to understand the role of biotic factors on pine invasion in natural communities.

This thesis is composed of three more chapters in which different hypotheses are tested to explain the observed pattern of tree invasion on the island. Chapter II is devoted to the impact of exotic deer herbivory on Pinaceae invasion on the island and entails analyzing deer preference patterns among native and exotic species. This study was conducted in collaboration with Dr. Relva and Dr. Simberloff. Chapter III analyses the importance of see predation in halting invasion with a series of experiment and observational studies and was conducted in collaboration with Dr. Simberloff and Dr. Relva. Chapter IV studies the role of mycorrhizal inoculation in the invasion process

with a series of field and greenhouse studies complemented with genetic analyses that aim to identify the fungal hosts and was conducted in collaboration with Dr. Horton and Dr. Simberloff. The goal of this dissertation was to test how different factors affect conifer invasion on the island, since invasions – as well as most ecological processes – are rarely controlled by single factors. Of course, other factors – many of them potentially fundamental – remain to be tested but I consider that the three factors discussed in this thesis are among the most important biotic factors controlling invasions. Future work should address the importance of other factors as well as their relative importance.

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## Appendix I:

## Tables and figures

**Table I-1.** Pinaceae species introduced to Isla Victoria, their common names, locations where native, areas where they are invasive, and areas where they are naturalized.

Shaded areas are species planted on the island that are reported to be invasive elsewhere. Only 12 species of Pinaceae that are invasive elsewhere were not introduced to the island: *Abies procera, Larix kaempferi, Pinus caribaea, P. clausa, P. elliottii, P. kesiya, P. koraiensis, P. luchuensis, P. muricata, P. patula, P. taeda, Tsuga heterophylla*. Code for countries: ARG-Argentina; Australia-AU; Belorussia-BRU; Canada-CA; Chile-CH, Czech Republic-CR, Germany-GE, Great Britain-GB; Hawaii-HA; Hungary-HU; Ireland-IR; New Zealand-NZ; South Africa-SA, Poland-PO, Russia-RU, Spain-SP, Lithuania-LI;. Codes for states and regions in USA California-CAL, Missouri-MS, Michigan-MIC, New York-NY, Southeast SE, Northeast-NE. (from Richardson and Higgins 1998, Simberloff *et al.* 2002, Richardson and Rejmánek 2004, Bustamante and Simonetti 2005).

| Species | common name | native location | invasive | naturalized |
|---|---|---|---|---|
| *Abies alba* | Silver fir | Europe | | GB, IR, NZ |
| *Abies balsamea* | Balsam fir | North America | | |
| *Abies bifolia* | Rocky Mountain alpine fir | North America | | |
| *Abies cephalonica* | Greek fir | Europe | | GB |
| *Abies chensiensis* | Shensi fir | Asia | | |
| *Abies grandis* | Grand fir | North America | GB | IR, Sweden |
| *Abies lowiana* | California white fir | North America | | |
| *Abies magnifica* | California red fir | North America | | |
| *Abies marocana* | Moroccan fir | Africa | | |
| *Abies nobilis* | Lindley noble fir | North America | | |
| *Abies nordmanniana* | Nordmann fir | Asia | | GB, NZ |
| *Larix decidua* | European larch | Europe | CR, GB, NZ | CA, IR, LI, NZ, USA (NE and NY) |
| *Larix laricina* | Tamarack | North America | | |
| *Larix leptolepis* | Japanese larch | Asia | | |
| *Picea abies* | Norway spruce | Europe | | AU, CR, GB, IR, PO, SP, USA (NE) |
| *Picea engelmannii* | Engelmann spruce | North America | | |
| *Picea glauca* | White spruce | North America | | |
| *Picea orientalis* | Oriental spruce | Asia | | |
| *Picea pungens* | Colorado blue spruce | North America | | USA (NE, NY) |
| *Picea sitchensis* | Sitka spruce | North America | GB, IR, NZ | |
| *Pinus banksiana* | Jack pine | North America | LI, NZ | BRU, PO, RU, USA ( NY) |

**Table I-1:** Continuation.

| Species | common name | native location | invasive | naturalized |
|---|---|---|---|---|
| *Pinus bungeana* | Lacebark pine | Asia | | |
| *Pinus canariensis* | Canary Island pine | Canary Islands | SA | AU |
| *Pinus contorta* | Lodgepole pine | North America | AU, GB, NZ | ARG, CH, RU |
| *Pinus densiflora* | Japanese red pine | Asia | | |
| *Pinus halepensis* | Aleppo pine | Asia, Europe | AU, NZ, SA | Israel, USA (CAL) |
| *Pinus jeffreyi* | Jeffrey pine | North America | AU | HA |
| *Pinus lambertiana* | Sugar pine | North America | | |
| *Pinus monticola* | Western white pine | North America | | ARG |
| *Pinus mugo* | Mugo pine | Europe | NZ | GB, LI, USA (NE), RU |
| *Pinus nigra* | Austrian pine | Europe | AU, GB, HU, NZ ; USA (MIC) | CR, LI, RU USA (NE) |
| *Pinus pinaster* | Cluster pine | Africa Europe | AU CH, GB, HA, NZ, SA, Uruguay | La Reunion |
| *Pinus pinea* | Italian stone pine | Europe | SA | AU, Mediterranean Basin, USA (CAL) |
| *Pinus ponderosa* | Ponderosa pine | North America | ARG, AU, CH, NZ | RU |
| *Pinus radiata* | Monterrey pine | North America | AU, CH, HA, NZ, SA, SP | GB |
| *Pinus rigida* | Pitch pine | North America | | Italy |
| *Pinus sabiniana* | Digger pine | North America | | |
| *Pinus strobus* | Eastern white pine | North America | CR, HU, NZ | BRU, Bulgaria, GER, GB, PO, RU, Ukraine |
| *Pinus sylvestris* | Scots pine | Asia, Europe | CA (Ontario), CH, NZ | ARG, IR, USA (NE; SE, NY) |
| *Pinus thunbergii* | Japanese black pine | Asia | | China, USA (NE) |
| *Pinus wallichiana* | Bhutan pine | Asia | | |
| *Pseudotsuga mensiezii* | Douglas fir | North America | ARG, Austria, Bulgaria, CH, GER, GB, NZ | CR, IR, USA (New York) |
| *Tsuga canadensis* | Eastern hemlock | North America | | Georgia (former USSR), PO, USA (MS) |

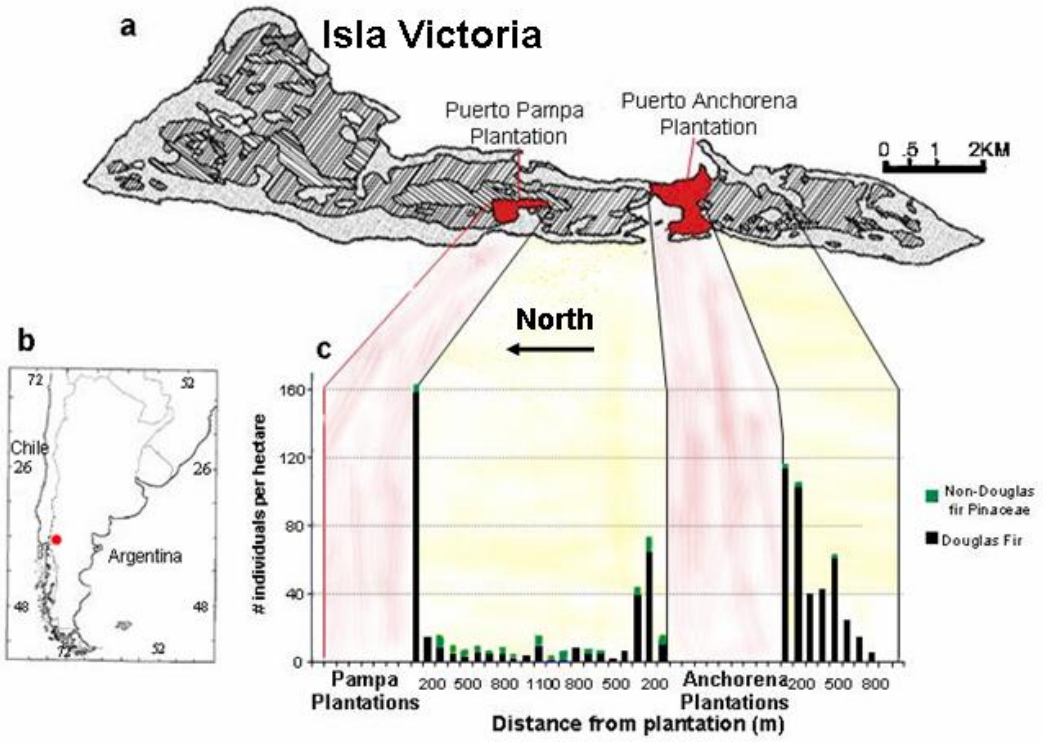


**Figure I-1.** Map of Isla Victoria with its geographic location and abundance of Douglas fir and other Pinaceae outside plantations.

a) Map of Isla Victoria with plantations of exotic Pinaceae in red (Puerto Pampa plantations and Puerto Anchorena plantations). b) Geographic location of the study area (red dot). c) Density of seedlings, saplings and adults of Douglas fir (*Pseudotsuga menziesii*) and other Pinaceae outside plantations from the Puerto Pampa plantations southward through the Puerto Anchorena plantations. Note that the species of Pinaceae that are not Douglas fir attain very low densities, and even Douglas fir reaches a density that is not enough to make it a dominant species (ca. 160 individuals per hectare).

# CHAPTER II.

## Enemy release or invasional meltdown? Deer preference for exotic and native trees on Isla Victoria, Argentina.

The following section is a slightly modified version of a paper to be published in the journal Austral Ecology:

Nuñez, M. A., M. A. Relva, and D. Simberloff. In Press. Enemy release or invasional meltdown? The role of exotic herbivores in pine invasion on Isla Victoria, Argentina. Austral Ecology.

The use of "we" in this part refers to my co-authors and me. As the lead author of this article I was responsible for this paper. My primary contributions to this paper included the design of the experiment, data collection and statistical analysis. I also wrote most of the paper.

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**Abstract**

How interactions between exotic species affect invasion impact is a fundamental issue on both theoretical and applied grounds. Exotics can facilitate establishment and invasion of other exotics (invasional meltdown) or they can restrict them by re-establishing natural population control (as predicted by the enemy-release hypothesis). We studied forest invasion on an Argentinean island where 43 species of Pinaceae, including 60% of the world's recorded invasive Pinaceae, were introduced ca. 1920 but where few species are colonizing pristine areas. In this area two species of Palearctic deer, natural enemies of most Pinaceae, were introduced 80 years ago. Expecting deer to help to control the exotics, we conducted a cafeteria experiment to assess deer preferences among

the two dominant native species (a conifer, *Austrocedrus chilensis*, and a broadleaf, *Nothofagus dombeyi*) and two widely introduced exotic tree species (*Pseudotsuga menziesii* and *Pinus ponderosa*). Deer browsed much more intensively on native species than on exotic conifers, in terms of number of individuals attacked and degree of browsing. Deer preference for natives could potentially facilitate invasion by exotic pines. However, we hypothesize that the low rates of invasion currently observed can result at least partly from high densities of exotic deer, which, despite their preference for natives, can prevent establishment of both native and exotic trees. Other factors, not mutually exclusive, could produce the observed pattern. Our results underscore the difficulty of predicting how one introduced species will affect the impact of another one.

## Introduction

Biological invasions can cause species extinction, habitat degradation, change in ecosystem function, and facilitation of further invasions (D'Antonio *et al.* 2001). Interactions between introduced species and natives can greatly affect invasions (see Richardson *et al.* 2000). Two hypotheses that entail interactions between exotic species have recently been advanced to explain the invasiveness of some exotic species. These are the enemy release hypothesis and the invasional meltdown hypothesis. The enemy release hypothesis states that exotic species experience decreased regulation by natural enemies (e.g., herbivores), resulting in increased abundance and distribution (Williamson 1996; Keane and Crawley 2002; Colautti *et al.* 2004; Agrawal *et al.* 2005). In invasional meltdown, synergy between exotic species facilitates their invasion and/or increases their

invasiveness and impact (Simberloff and Von Holle 1999; Simberloff 2006). Some exotic species could be subjected to both factors (released from enemies and facilitated by other invaders). Also, it is possible that introduced herbivores can have either effect on invasive plant species (serving either as biocontrol agents or as facilitators) depending on the details of the local ecosystem.

Herbivores influence diverse ecological processes, including biological invasion (Bellingham and Coomes 2003; Chauchard *et al.* 2006). In forest systems, wild ungulates such as deer damage trees by browsing, stripping bark, and fraying (Gill 1992b). Browsing is the most important damage, affecting sapling growth and survival (Hester *et al.* 2000; Gill and Beardall 2001). Evidence for the mechanisms by which ungulates affect invasion by non-native plants is often inconclusive (Parks *et al.* 2003). Herbivory by mammals may contribute to the success or failure of plant invasion by selective attacks (Maron and Vila 2001; Parker *et al.* 2006). If they affect native plants more heavily and reduce their abundance, deer would benefit exotic plant species by competitive release – which can be seen as evidence for invasional meltdown. On the other hand, if deer preferentially browse exotic plants, they could prevent invasion, which would support the importance of enemy release in determining extent of an invasion.

On an Argentinean island with old plantations of many exotic tree species adjacent to native forest, only a few species have begun to invade (Simberloff *et al.* 2002). We sought to determine the effect of exotic herbivores, in this case Palearctic deer, on the invasion process. On the one hand, deer could be driving

or contributing to the observed low invasion rates by Pinaceae on Isla Victoria if deer damage exotic conifers more than native species. However, if deer prefer native species over exotics, they could aid establishment of exotic seedlings, an impact that can be exacerbated by their disturbance of the soil. The relative impacts of deer on native and exotic tree species remain unexplored.

Simberloff *et al.* (2002, 2003), based on field observation of native and exotic trees in the area, suggested that deer could be halting invasion of exotic trees by selectively browsing on them. Such a hindrance to pine establishment has been observed in other areas where pines are introduced (Richardson *et al.* 1994) and where they are native (Rogers *et al.* 2006). Thus, our hypothesis is that exotic deer impede invasion by exotic trees by damaging them more than their native competitors.

## Methods

### Study system

Isla Victoria, in Nahuel Huapi National Park, Argentina (Fig. II-1), is 20 km long and 4 km across at its widest point. In 1902 the island began to suffer major damage from logging, cattle ranching, and fires, which affected 50-60% of the island. With the establishment of the national park in 1934, such activities decreased. Today most of the island is covered by either primary or substantial secondary forest dominated by *Nothofagus dombeyi* and *Austrocedrus chilensis* trees, but several roads, trails, buildings, and abandoned pastures remain.

In 1917 Palearctic deer were introduced: *Cervus elaphus* (elk or red deer), *Dama dama* (fallow deer), and *Axis axis* (axis deer). In 1962, combined red and fallow deer density was estimated at 40 $km^{-2}$ (Anziano 1962) and more recently at 26 $km^{-2}$ (Relva unpublished data); axis deer disappeared. These densities are very high compared to normal densities in native areas, which range from 6 to 20 deer $km^{-2}$ (Mayle *et al.* 2000, Scott *et al.* 2000) or compared to those in other areas where deer are exotic, such as New Zealand, where densities of 16 $km^{-2}$ are considered high (Nugent *et al.* 2001). Native deer *Pudu pudu* and *Hippocamelus bisulcus* have become very rare in the region, probably because of human activities (Vazquez 2002).

In 1925, the Argentine government established a tree nursery on Isla Victoria. A few species introduced then had already been introduced beginning in 1910 (Koutché 1942, APNA 1988). The last plantings by the government were in 1939. At least 73 conifers – including 60% of the world's known invasive conifer species – and 62 broad-leaved species were planted, including species from every continent but Antarctica. Included were 43 species of Pinaceae, including 17 of the 28 species of Pinaceae that Richardson and Rejmánek (2004) cite as having invasive qualities. There has been no recent survey of which species remain on Isla Victoria, but at least 50 are apparent to casual observation (Simberloff *et al.* 2002), occupying an extensive area (Fig. II-1). Of the 22 species of the genus *Pinus* ca. 20 years after their introduction, 19 were well established and most were producing viable seeds (Barrett 1952). However, only a few species have dispersed beyond the plantations; most are present in very low densities if at all

outside the plantations (Simberloff *et al.* 2002, Fig. II-1). Among introduced Pinaceae, Douglas fir (*Pseudotsuga menziesii*) is by far the most widespread.

## Field experiment

### Selective browsing by deer

We conducted an experiment to determine whether deer preferentially browse on native (*Austrocedrus chilensis* and *Nothofagus dombeyi*) or introduced *(P. menziesii* and *Pinus ponderosa)* tree species. *A. chilensis* and *N. dombeyi* are the only dominant trees in this region; the exotic *P. menziesii* reaches high densities near plantations but is rare far from them, and *P. ponderosa* is found outside plantations but is always rare (Simberloff *et al.* 2002). We collected saplings from five different locations on the island to offer an array of individuals with nutritional composition (Bergman *et al.* 2005) similar to what deer find in nature.

In late winter – early spring 2005 (August 31$^{st}$ until October 1$^{st}$ ), in 45 feeding stations we randomly placed one sapling, ~1 m tall, of each of the four tree species in each corner of a 1.5 x 1.5 m square. We distributed feeding stations from the Puerto Pampa plantations southward through the Puerto Anchorena plantations in the same area where Simberloff *et al.* (2002) conducted their survey of exotic conifers (Fig. II-1). We used saplings uneaten by deer, to avoid possible plant responses to herbivory that can affect deer preference. Immediately before the experiment, we transplanted the saplings into pots 40 cm in diameter and 50 cm high and planted them the same day, burying the pots. We measured deer preference every two weeks during the first 10 weeks after the experiment started. We observed no ill effects on most planted trees that were not

browsed by deer, and at stations where at least one tree showed signs of stress (e.g., low turgor) we ceased measurement. We believe there was little effect on palatability or preference owing to experimental conditions. We used only the feeding stations that were not attacked by other animals or affected by human activities. At the end of the experiment, we were able to collect information from 38 of the initial 45 stations. No other animals on this island would browse on these saplings.

To quantify browsing preference, at each feeding station we recorded, for each sapling, the number of branches browsed divided by the total number of branches, and the type of deer damage (whether on lateral or apical shoots). We also recorded the number of saplings browsed and number of individuals with browsed apical shoots. Ten weeks after initiating the experiment we recorded the percentage of browsed branches per species to account for change in deer preference when the preferred species had been browsed.

**Statistical analyses**

We analyzed the data using logistic regression (for binomial variables) and Poisson regression (for percentages and counts) with the GENMOD procedure of SAS 9.1 (SAS 2003). We used species as a fixed effect and the feeding stations as a random effect in our model. These assignments accurately model the correlation found within feeding stations. These models account for lack of independence between the different saplings in the feeding stations, a common problem in this type of experiment, though most similar studies do not use this type of model (Mangeaud and Videla 2005).

## Results

Deer browsed more heavily on the natives *Austrocedrus chilensis* and *Nothofagus dombeyi* than on the exotic conifers *Pseudotsuga menziesii* and *Pinus ponderosa*. In terms of the number of individuals browsed on the first visit ($X^2$ = 21.45; P<0.001; Fig. II-2 A), percent of browsed branches ($X^2$ = 21.45; P<0.001; Fig. II-2 B), number of individuals with browsed apical shoots ($X^2$ = 24.02; P<0.001; Fig. II-2 C), and percent of individuals browsed at the end of the experiment ($X^2$ = 28.58; P<0.001; Fig II-2 D), both natives were preferred over both exotics. There were no significant differences between the two native trees or between the two exotics (in all cases $X^2 \leq 3.51$, P> 0.05).

## Discussion

Contrary to our hypothesis, we found that exotic deer preferentially browsed on native rather than exotic species, a preference that could potentially aid invasion by exotic pines, perhaps generating invasional meltdown. Despite the short term nature of this study and the limited number of species studied, we observed that deer preference was much greater for native tree species than for exotic conifers in terms of the number of browsed individuals and intensity of browsing. Deer are known to influence survival and growth of plant species and can strongly alter dominance patterns through selective browsing (Pastor *et al.* 1993, Zimov *et al.* 1995, Gill and Beardall 2001), and deer herbivory can suppress tree growth, limit competitive abilities, and affect tree survival rates

(Gill, 1992a; Ueda *et al.* 2003). Though browsing preference need not mean negative effects on tree growth, owing to the ability of some plants to compensate for lost tissues (McNaughton 1983), in our study areas, exotic deer have been shown to affect sapling growth and tree seedling density of natives (Veblen *et al.* 1989; Relva and Sancholuz 2000).

Differences in abundance between natives (abundant) and exotics (rare) could explain the observed patterns if deer foraging behavior depends on the relative abundance of plants. However, we think this is not a determining factor in this study, given the strength of the observed pattern of preference and the fact that there is evidence for frequency-independent forage selection by deer (Chevallier-Redor *et al.* 2001).

In New Zealand, as on Isla Victoria, deer and Pinaceae are both introduced. Introduced deer in New Zealand have caused a rapid decline of palatable tree, herb and shrub species (Allan et al 1984), but its effects are less clear on species that are avoided or at least not preferred, such as *Nothofagus* (Forsyth *et al.* 2002). Several researchers have found no evidence (Nugent 1990), of little evidence (Husheer and Frampton 2005) of negative effects of introduced deer on establishment and growth of *Nothofagus* seedlings, while others have found strong evidence (Allen and Allan 1997; Allan *et al.* 1997; Husheer *et al.* 2003; Husheer and Robertson 2005) of such effects.

Also in New Zealand, pine species constitute a small but noteworthy proportion of the diet of exotic deer (Nugent 1990, Nugent *et al*. 2001). The

different levels of tree invasion found in the two areas (Richardson and Higgins 1998, Simberloff *et al.* 2002, Buckley *et al.* 2005; Bustamante and Simonetti 2005, Williams and Wardle 2005) suggest that deer may play a similar role in pine invasions of Isla Victoria and New Zealand, but that factors impeding the invasion on Isla Victoria are not operating in New Zealand.

Deer can be important in regulating regeneration of native (Bellingham and Allan 2003; Gill 1992a) and exotic tree species (Richardson and Bond 1991). Because deer attain very high densities in our study area compared to those where it is native, the observed high intensity of browsing (Barrios-Garcia 2005) could limit both exotics and natives. If deer had achieved somewhat lower density, they might have promoted Pinaceae invasion because of the currently much higher abundance of native species. This idea is consistent with the hypothesis of Richardson *et al*. (1994) that low or high levels of herbivore pressure could control spread of introduced pines but intermediate levels could promote invasion.

Observational studies of deer browsing on Isla Victoria (Barrios-Garcia 2005) showed high preference for native *A. chilensis* saplings in relation to their availability relative to the preference for *N. dombeyi*. Barrios-Garcia surveyed saplings of different species in randomly selected plots. She found that 81% of individuals of *A. chilensis* were browsed versus 36% of individuals of *N. dombeyi*. Nevertheless, fecal composition analyses reveal that both *A. chilensis* and *N. dombeyi* constitute important food items during the year (32.3% of their total diet), and they are especially important during winter – 46.2% of their total

diet (22.2 % *A. chilensis* and 24% *N. dombeyi*) (Relva and Caldiz 1998). Unfortunately, no data are available on introduced tree species in deer diets on Isla Victoria. Barrios-Garcia also collected data on browsing on exotic conifers, finding that 48% of the individuals recorded were browsed by deer. Despite the large dissimilarities between the sample sizes of exotic and native tree species (27 and 2277, respectively) caused by the local rarity of the introduced conifers, these data support the idea that lower densities of deer could generate a release from herbivory for the exotic tree species.

The relative lack of tree invasion found on Isla Victoria cannot be explained entirely by herbivory by exotic deer. Deer might have been seen as an inadvertent case of biological control, but actually they are having the opposite impact through selective herbivory on natives and also perhaps by other types of disturbances such as ground alteration (Richardson *et al.* 1994). For example, trampling has been observed to favour establishment of exotic conifers at Isla Victoria (Simberloff *et al*. 2002) and elsewhere (Richardson and Bond 1991). Our results coupled with information from other studies suggest that deer could potentially aid invasion of exotic conifers and that this invasion may presently be halted by the high densities of deer found in the areas.

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## Appendix II: Figures

**Figures**

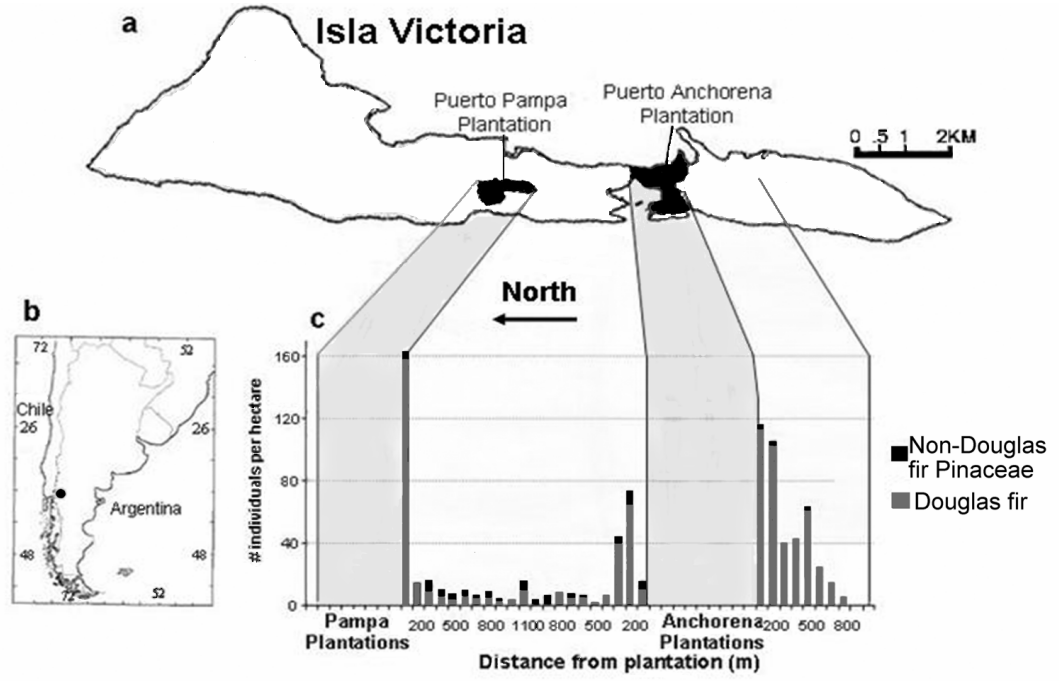
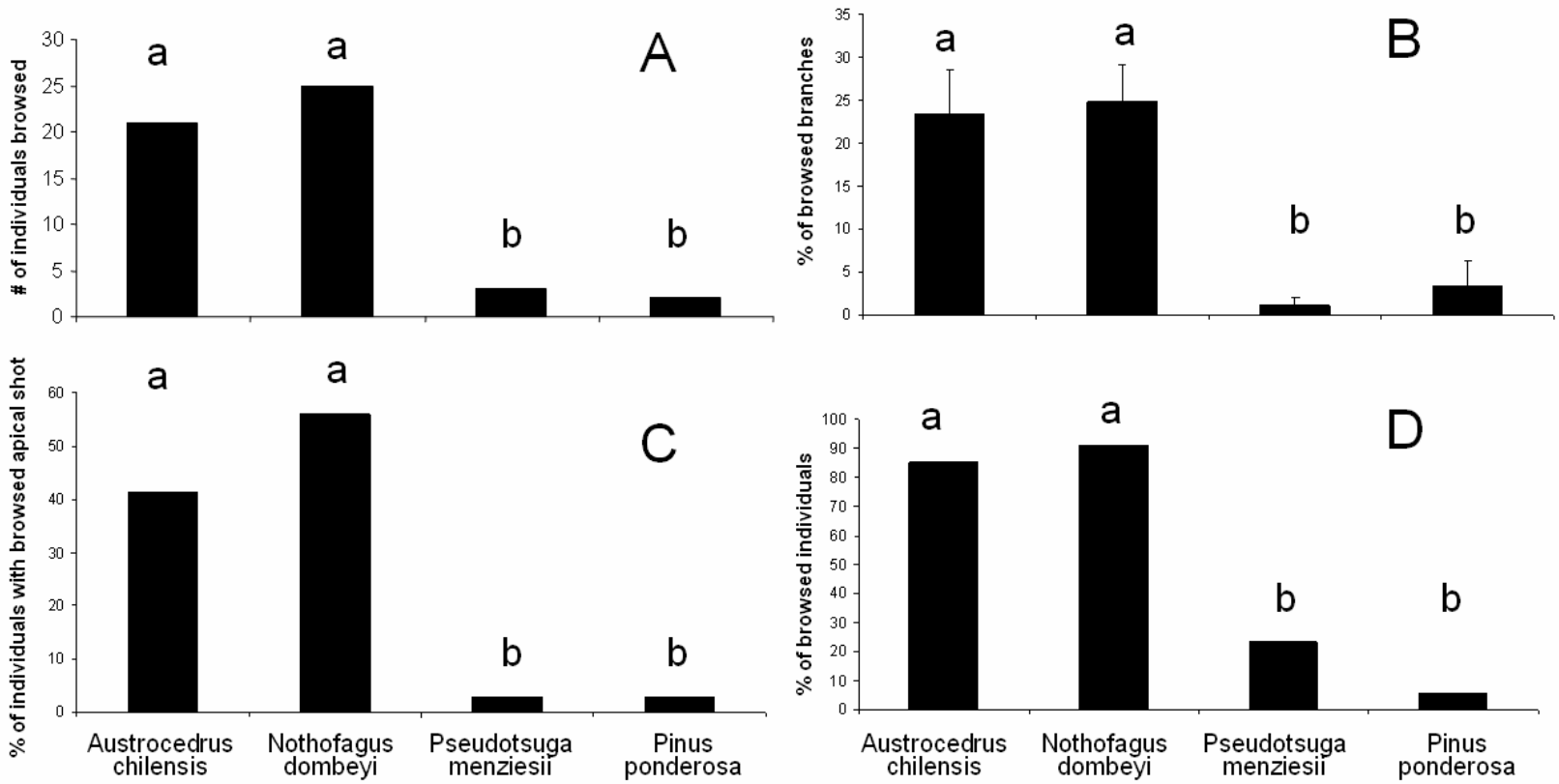



**Figure II-1.** Isla Victoria with its geographical location and abundance of exotic conifers outside plantations.

a) Map of Isla Victoria with plantations of exotic Pinaceae in black (Puerto Pampa and Puerto Anchorena plantations). b) Geographic location of the study area (black dot). c) Density of seedlings, saplings and adults of Douglas fir (Pseudotsuga menziesii, in grey) and other Pinaceae (in black) outside plantations from the Puerto Pampa plantations southward through the Puerto Anchorena plantations found after a detailed survey using 10 m wide transects distributed every 100 m, parallel to the plantations (see Simberloff *et al.* [2002] for a detailed description of the methods).

**Figure II-2.** Results of the cafeteria experiment for the two native (*A. chilensis* and *N. dombeyi*) and two exotic (*Pseudotsuga* and *Pinus*) tree species at the 38 stations attacked by deer.

Different letters represent significant differences for an α of 5%. **a**) Individuals browsed at the time of the first deer visit (first time when browsing was recorded in the different stations). **b**) Percent of browsed branches at the time of first deer visit. **c**) Percent of individuals with browsed apical shoot at the time of the first deer visit. **d**) Percent of individuals with browsed branches at the end of the experiment (10 weeks).


# of individuals browsed
30
25
20
15
10
5
0
a
a
b
b
A
% of browsed branches
35
30
25
20
15
10
5
0
a
a
b
b
B
% of individuals with browsed apical shot
60
50
40
30
20
10
0
a
a
b
b
C
% of browsed individuals
100
90
80
70
60
50
40
30
20
10
0
a
a
b
b
D
Austrocedrus chilensis
Nothofagus dombeyi
Pseudotsuga menziesii
Pinus ponderosa

# CHAPTER III.

## Seed predation as a barrier to alien conifer invasions

The following section is a slightly modified version of a paper to be published in the journal Biological Invasions

Nuñez, M. A., D. Simberloff, and M. A. Relva. In Press. Seed predation as a barrier to alien conifer invasions. Biological invasions.

The use of "we" in this part refers to my co-authors and me. As the lead author of this article I was responsible for this paper. My primary contributions to this paper included the design of the experiment, data collection and statistical analyses. I also wrote most of the paper.

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**Abstract**

Interactions between exotic plants and animals can play a major role in determining success or failure of plant introductions. Seed predation has been seen as important in explaining biotic resistance to plant invasion, but this hypothesis has rarely been tested. We studied seed predation on exotic forest plants on an island in Patagonia, Argentina where 43 pine species, including 60% of the world's known invasive Pinaceae, were introduced ca. 80 years ago, but where exotics attain relatively high densities only near the original plantings. To test if seed predation limits exotic conifer establishment in this area, we compared seed predation in areas close to plantations (colonized by exotics) and far from them (not invaded). Seeds of exotics were preferred over seeds of native species, possibly because exotic seeds are bigger. Predation was more intense in areas far from plantations than in areas close to

them, substantially reducing the chances of exotic seed establishment. Using automatic cameras, we found that both rodents and birds preyed on exotic seeds. This study suggests that native seed predators can be an important component of biological resistance to plant invasion.

## Introduction

Biological invasions are a leading cause of global change: they eliminate native species, degrade habitat, change ecosystem function, and facilitate further invasions (D'Antonio *et al.* 2001). However, most introduced species fail to establish and spread (Williamson and Fitter 1996). Among many hypotheses proposed to explain the success or failure of invasive species are two prominent ones, with opposite predictions: the enemy release hypothesis and the biotic resistance hypothesis (Lockwood *et al.* 2007). The enemy release hypothesis states that exotic species in their new ranges are less strongly regulated by natural enemies (e.g., herbivores, seed predators, parasites), resulting in an increase in their abundance and distribution (Agrawal *et al.* 2005, Colautti *et al.* 2004, Keane and Crawley 2002, Williamson 1996). By contrast, the biotic resistance hypothesis (Elton 1958, Levine *et al.* 2004; Parker and Hay 2005) states that native interactions with exotics can impede invasions.

For plants, seed predation is proposed as a major impediment to establishment (Orrock *et al.* 2006). Seed destruction can reduce and even stop recruitment and expansion of native species (Jules and Rathcke 1999, Maron and Simms 2001, Ostfeld *et al.* 1997) and has been suggested as a potential mechanism controlling invasion and

spread of exotic plant species (Maron and Vilá 2001). However, its importance in controlling invasions is not yet clear owing to the lack of direct evidence that seed predators halt invasions (Bossard 1991, Hoffmann and Moran 1998, Parker 2000, Vila and Gimeno 2003). In theory, changes in degree of seed predation can promote or halt the establishment and spread of introduced species depending on its relative effects on natives and exotics. If an exotic species suffers less seed predation in its introduced than in its native range, this can be evidence for enemy release (see Hierro *et al.* 2005). On the other hand, seed predators may prefer exotic seeds over native seeds and thus restrict exotic establishment, a form of biotic resistance.

On an Argentinean island with plantations (ca. 80 years old) of many exotic tree species adjacent to native temperate forest, only a few exotic species have begun to invade, and they are found in high densities only near plantations (Simberloff *et al.* 2002). We sought to determine the effect of seed predation on conifer invasion. For conifers, Castro *et al.* (2002) showed that seed predation by rodents can limit the distribution of *Pinus sylvestris* in its native range. Also, post-dispersal predation has been recognized as a major source of seed loss in temperate forests (Caccia *et al.* 2006, Diaz *et al.* 1999, Schnurr et al. 2004).

In northwestern Patagonia, rodents consume seeds of exotic Pinaceae at very high rates (Caccia and Ballare 1998). A tentative hypothesis for the pattern observed by Simberloff *et al.* (2002) is that plantations and areas influenced by plantations offer less suitable habitat for native animals and contain fewer seed predators, as has been found in other areas (see Estades 1994, Lindenmayer and Hobbs 2004, Muñoz and

Murúa 1989, 1990, Sykes *et al.* 1989). Another potentially important factor influencing the pattern of invasion on Isla Victoria is that these seeds are wind-dispersed and there is no evidence of secondary dispersal by rodents in this area (Caccia and Ballare 1998, Diaz *et al.* 1999). Thus, seed predators could be contributing to the observed patterns of few established populations outside the plantations and a rapid decrease in number of individuals of species that do establish with increasing distance from plantations. Our hypothesis is that post-dispersal seed predation limits establishment of exotic Pinaceae. We predict that seed predation will be more intense in areas far from plantations of exotics trees and that exotic seeds will be preferred over the abundant seeds of native trees. Here we present results from two experiments and an observational study designed to test this hypothesis.

## Methods

### Study system

Isla Victoria (40° 57 S, 71° 33 W), in Nahuel Huapi National Park, Argentina (Fig. III-1), is 20 km long and 4 km wide. Beginning in 1902, Isla Victoria experienced logging, cattle ranching, and fires, which together affected 50-60% of its total area. These activities decreased when Nahuel Huapi National Park was established in 1934, although there was a functioning plant nursery and cattle ranching into the 1960s (Basti 1988). Today Isla Victoria is dominated by primary or secondary forest of coihue (*Nothofagus dombeyi*) and ciprés (*Austrocedrus chilensis*) trees with a dense understory vegetation composed mostly of shrubs, but several roads, trails, buildings, and abandoned pastures remain (Simberloff *et al.* 2003).

The Argentine government established a nursery on Isla Victoria in 1925, planting forestry and fruit trees potentially suitable for the region. A variety of exotic species were introduced beginning in 1910 (APNA 1988; Koutché 1942) and planted until 1939. At least 73 conifers, including 60% of known invasive conifer species, and 62 broad-leaved species were on the island. Among the species planted were 21 species of *Pinu*s, including 9 of the 12 species that Rejmánek and Richardson (1996) cite as having invasive qualities, and 43 species of Pinaceae, including 17 of the 28 species of Pinaceae for which Richardson and Rejmánek (2004) found evidence of invasiveness somewhere in the world. Despite a large introduction effort, only a few species have been able to disperse from where they were initially planted, and most that have dispersed attain very low densities (Simberloff *et al.* 2003; Simberloff *et al.* 2002) (Fig. III-1).

## Experiments on seed predation

### Seed removal

With a seed-removal experiment, we compared seed predation in native habitats in areas containing established exotic species adjacent to plantations (< 100 m from a plantation boundary) and areas far from plantations with low densities of invaders (>1500 m from a plantation boundary). We conducted the experiment during winter (June – August 2005) after seed release to explore the period during which rodents are famished and seed search is more intense. We set 60 seeds per species (5 species) per treatment (near and far). We conducted this study from the Puerto Pampa plantations southward through the Puerto Anchorena plantations (Fig. III-1). Seeds were haphazardly placed over the ground in different sites (60 sites per treatment per

species). We placed only one seed per site to mimic conditions after long distance dispersal and also to avoid density-dependent attacks. Long distance dispersal is uncommon but is a key mechanism for pine invasion (see below). We studied five different species – three exotics that are known to be highly invasive elsewhere (*Pseudotsuga menziesii, Pinus contorta* and *Pinus ponderosa*) and the two dominant natives (*Austrocedrus chilensis* and *Nothofagus dombeyi*) – to test if local seed predators prefer exotic seeds. We expect such a preference because exotic seeds are bigger and thus more evident and attractive than those of the native species (Reader 1993). We recorded seed removal 20 days after we set them out. Seeds were glued to wooden flat stakes (1 cm by 10 cm) with a nontoxic odorless adhesive and manipulated with forceps to avoid scent contamination. Because seeds were glued to a stake, we can assume missing seeds were preyed upon.

**Seed predator exclosures**

To determine the overall effect of seed predation on pine early establishment, we set up rodent- and bird-exclosures, constructed of metal-mesh (1 x 1 cm, with 0.06 cm wire width), and controls (without the fence). We analyzed seed predation in three species (*Pseudotsuga menziesii, Pinus contorta* and *Pinus ponderosa*). Treatments consisted of a factorial combination of three factors: seed species, distance, and exclosure. For each species and at each location (adjacent to and far from plantations), we set up 50 10-cm by 10-cm by 15-cm tall exclosures. Each wire exclosure was embedded 5 cm into the soil to avoid rodent penetration and firmly fixed to the ground. For controls a 10 cm x 10 cm x 6 cm tall wire fence without a covered top was embedded 5 cm into the ground to minimize losing seeds to runoff and to mimic

treatment conditions as closely as possible. We planted one seed per plot per species in 50 randomly selected locations far from (>1500 m) and adjacent to (<100 m) plantations. Seeds were deposited on the surface on the plots, but with time they were naturally covered by litter or soil. Every 15 days for the first four months of the growing season (September – December 2005) and every month thereafter until June 2006, we surveyed seedling emergence in all plots, because we expected variance in time of germination of seeds throughout the year. We measured emergence only once a month from January to June because emergence rates diminished notably, although we found some newly emerged seedlings. In areas adjacent to plantations we also measured seedling establishment at sites where we did not add seeds, to control for seed addition from neighboring exotic trees. To this end we randomly selected an area the same size as the established plots, in which we recorded seedling emergence. We assessed maximum seedling emergence rates using data from a companion study in which we planted 400 seeds of each species used in this experiment in 40 pots (1 liter each) and grew them in a greenhouse with soil from the island and water *ad libitum* (see chapter IV). Seeds were cold-stratified to mimic natural germination conditions and manipulated using forceps to avoid scent contamination.

**Identification of seed predators**

We haphazardly distributed forty automatic cameras (CamTrakker Wildlife Pro Camera®) with movement sensors in areas both adjacent to plantations and far from them to record seed predator identities. Each system consisted of a fully automatic camera combined with a passive infrared motion detector. We arranged four cameras per tree species. We aimed four cameras at bait stations consisting of one gram of

seeds of each of five species (*Pseudotsuga menziesii, Pinus contorta, P. ponderosa, Austrocedrus chilensis* and *Nothofagus dombeyi*) in two different areas close to and far from plantations (4 cameras per species, two areas, five species; total = 40 cameras). We left the cameras for approximately one week during spring. We were able to identify birds to species and the presence of rodents. Camera resolution prohibited us from distinguishing rodent species, although size and morphological characteristics enabled us to identify likely candidates.

We did not study the role of arthropods on seed predation, because we did not observe any seed-predator arthropods on the island in any of our multiple visits to the site in winter or early spring (possibly owing to cold temperatures), when seed predation is most important in determining plant establishment. In this region, insect abundance is always extremely low during this period (P. Sackmann, Pers. Comm.), and rodents are believed to be the main post-dispersal predators in the system (Bustamante 1996, Caccia *et al.* 2006).

**Statistical analyses**

We analyzed results of the seed removal experiment using binary logistic regression, since our response variable was binary (eaten or not eaten) and the independent variables (fixed effects) were categorical; distance was binary and species had five categories (Hosmer and Lemeshow 2000). We also tested for differences between native and exotic species and differences among the different species, using contrast tests in the logistic procedure of SAS 9.13 (SAS 2003). In these models we analyzed both main effects and interaction effects. In the seed exclosure experiment

we tested for the effect of species (three categories), exclosures (presence-absence), and location (close to and far from plantations) on seedling establishment using binary logistic regression. We tested for the main effect and for interactions between different variables (Logistic Procedure in SAS 9.13 (SAS 2003)).

## Results

### Seed removal

Predation rate was higher at areas located far from the plantations than at sites adjacent to them ($\chi^2$ = 14.94, DF = 1, P< 0.001) (Fig. III-2). Also, seeds from exotic species were preferred over seeds from native species ($\chi^2$ = 24.77, DF = 1, P< 0.001). Seed predators preferred seeds in the following order: *Pinus ponderosa* (exotic), *P. menziesii* (exotic), *P. contorta* (exotic), *A. chilensis* (native), and *N. dombeyi* (native) (Fig. III-2). Seed predation was closely related to seed mass; seeds with higher masses were preferred (Table III-1). The interaction between species type and distance from the plantation was not significant ($\chi^2$ = 4.26, DF = 4, P >0.37).

### Seed predator exclosures

We found significant effects of distance from the plantation ($\chi^2$ = 7.85, DF = 1, P < 0.005) and the presence of a protective cage ($\chi^2$ = 19.93, DF = 1, P < 0.001) on seedling emergence. However, we found no differences among tree species in the number of seedlings emerged ($\chi^2$ = 2.91, DF = 2, P > 0.232), although in areas far from plantations the trend was consistent with the results of the seed removal experiment. The interaction between the two variables (distance and cage) was marginally significant ($\chi^2$ = 3.00, DF = 1, P > 0.083) (Figs. III-3 and III-4), and the interactions between species and distance and between species and presence of a

protective cage were not significant ($\chi^2 = 0.55$, DF = 2, P > 0.76; and $\chi^2 = 1.49$, DF = 2, P > 0.47 respectively). Also, the 3-way interaction among the variables was not significant ($\chi^2 = 0.568$, DF = 2, P > 0.753).

Seedling emergence outside cages was four times higher in areas adjacent to plantations than in areas far from them (27 vs. 6 seedlings, respectively), in accord with the results of the seed removal experiment. Emergence in cages was also higher in areas adjacent to plantations than in areas far from them (55 vs. 33 seedlings, respectively) (Fig. III-3). This change in proportion of seedling emergence may explain the marginal significance of the interaction terms. In areas near plantations we found only five emerged seedlings in the 300 plots without seed addition, suggesting a minimal effect from natural seed addition in our experiment. From the companion greenhouse study, we obtained seedling emergence rates of at least 85% for all three species.

The seed removal experiment and the seed predation experiment produced similar results. Despite the differences in methods, seed predation was more intense in areas farther from plantations and heavier seeds were preferred over lighter ones (Figs. III-2 and III-4).

**Identification of seed predators**

Using cameras, we identified two bird species as seed predators: *Scelorchilus rubecula* and *Phrygilus patagonicus.* Owing to camera limitation, we were unable to identify rodents to species. However, based on the size and morphology of the

rodents in the photographs and on previous studies in the area, rodent seed predators were most likely *Abrothrix olivaceus* and *Oligoryzomys longicaudatus* (cf. Caccia *et al.* 2006). We were able to get pictures of birds or rodents consuming seeds of all species in the different areas studied except for seeds of *Nothofagus*, which were the least preferred. In areas far from plantations we obtained photographs of 14 different individuals consuming seeds (9 rodents and 5 birds); in areas adjacent to plantations we obtained photographs of 7 individuals (3 rodents and 4 birds).

## Discussion

Seed predation has been suggested as an important control of exotic plants, especially when these plants are not superabundant (Maron and Vilá 2001). Our data suggest that seed predators limit the establishment of exotic conifers and retard invasion in our study system. Predation was more intense in uninvaded areas than in areas with high densities of exotic conifers, pointing to a mechanism for the current lack of invasion.

Plantations are not well suited for native animals in this region, and local animals are rare inside plantations. In the region of our research, studies have shown a pattern of low number of individuals in plantations for rodents (Muñoz and Murúa 1989, 1990) and birds (Schlatter and Murúa 1992), especially granivorous birds (Estades 1994). This habitat difference may cause the pattern of less predation in areas near plantations. If plantations had not lessened the presence of seed predators, we would have expected many seeds to have been consumed there as a consequence of a numerical response by local rodents and birds to the abundant exotic seeds.

Rejmánek and Richardson (1996) found that seed size contributed to invasiveness for pine trees. They suggested that species with smaller seeds could be more invasive because they produce more seeds and have higher initial germinability, shorter periods of chilling to overcome dormancy, and higher relative growth. In a subsequent study of conifers, Richardson and Rejmánek (2004) also suggested that species with very large seeds can be invasive only if they find a local vector to disperse their seeds, which is an uncommon event. Our study suggests that seed size can also be related to chances of seed predation, because seed size is known to affect preference by seed predators (Reader 1993, but see Moles *et al.* 2003, Moles and Westoby 2003). Despite the fact that seed predation was related to seed mass, other seed characteristics such as protective tissue or chemical defenses could be playing an important role.

Species characteristics, such as their dispersal abilities, are a key part of the invasion process. Colonization can be dispersal-limited at relatively small scales (Cadenasso and Pickett 2001, Pauchard and Alaback 2004). Seed dispersal is well-studied in pines (Lanner 1998). A very small percentage of seeds is known to disperse more than 100 m, but such relatively rare events of long-distance dispersal are extremely important for pine invasion (Higgins and Richardson 1999). Our results show that seed dispersal may play an important role, given that a small but noteworthy number of seedlings were able to emerge far from plantations (Fig. III-3). However, conifer invasion in our system seems not to be limited only by dispersal, given the different levels of seed predation and seedling emergence at different distances from seed sources.

More seedlings emerged close to plantations than far from them in caged treatments. This result could be due to differences in abiotic characteristics of the different areas, soil characteristics, and/or characteristics of the soil biota that could affect germination rates or early survival. For example, we know that pines need ectomycorrhizal fungi to survive, and when ectomycorrhizal plants are established, the primary inoculation source is thought to be existing hyphal networks associated with established plants (Newman 1988; chapter IV). Establishment of exotic Pinaceae around plantations is then limited to the root zone of the plantation trees, where seedlings can tap the mycelial network. Establishment beyond the root zone of trees is then limited by the presence of spore inoculum, but there is evidence of poor dispersal ability of fungal species outside Pinaceae plantations (Davis *et al.* 1996, Lamb 1979, Mikola 1953). Plantations can modify soil properties like pH and water content (Jackson *et al.* 2005) that could facilitate seedling establishment near plantations. These finding are evidence of the complexities of the invasion process, which is probably in most cases affected by multiple factors.

Seedling herbivory has been proposed as an important factor limiting pine establishment and invasion (Fuentes and Etchegaray 1983, Kruger *et al.* 1989, Richardson *et al.* 1994). Seedling herbivory could have affected our results, as seedlings in exclosures were less likely to be attacked than seedlings outside them. However, because we took measurements frequently, it is unlikely that herbivory on seedlings could have produced a strong difference. Also, results from a companion study on Isla Victoria on the role of seedling herbivory on pine invasion show

relatively low rates of seedling herbivory. Only 8% of individuals of *Pinus ponderosa* and *Pseudotsuga menziesii* were attacked by herbivores during seven months starting in late winter (when seedling emergence is highest) in areas adjacent to and far from plantations (M.A. Nuñez unpublished data).

Abiotic factors such as climate can play an important role in controlling invasion of exotic conifers (Richardson and Bond 1991). However, the large number of species introduced, many of them from areas with similar climates to that of Isla Victoria (Critchfield and Little 1966) or that are successfully invading areas similar to Isla Victoria (Richardson and Rejmánek 2004), suggests that these abiotic factors cannot fully explain the observed invasion pattern. Also the fact that many of these species are well-adapted to the area and attain remarkable growth rates in silviculture in the region (Schlichter and Laclau 1998) supports the idea that abiotic factors cannot completely explain invasion patterns on Isla Victoria.

It is important to note the limitations of this study. Seed predation and production show marked inter-annual fluctuations (Schnurr *et al.* 2004; Wilson *et al.* 2007). Therefore, our results, obtained in a single year, can yield limited information, since we cannot assess how this pattern would change between years. However, we can say, based on the data collected, that seed predation has probably affected invasions by our study species in this area, especially in areas distant from plantations, therefore acting as a barrier for conifer invasion.

Most studies on biological invasions are conducted on species that are already invading some area. By contrast, this study focused on species that one might have expected to invade – given their traits and the area characteristics – but that have not yet spread far from the point of introduction. This kind of system, in which species are not yet invading, can yield valuable information about what factors are limiting invasions rather than what factors are promoting them, and this information can aid land managers. This study suggests seed predation – a factor often proposed as important to plant invasion but one seldom tested – can be an important factor producing biological resistance and retarding plant invasion.

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## Appendix III:

## Tables and figures

**Table III-1.** Approximate weight and average seed predation rates for the species studied in the seed removal experiment.

Sources: Caccia *et al.* 2006, Sarasola *et al.* 2006. Exotic species are denoted by *.

| Species | Average predation (%) | Seed weight (mg) |
|---|---|---|
| *Nothofagus dombeyi* | 6.752874 | 2.4 |
| *Austrocedrus chilensis* | 21.19048 | 4.3 |
| *Pinus contorta* * | 28.44633 | 5.7 |
| *Pseudotsuga mensiezii* * | 36.90678 | 13.7 |
| *Pinus ponderosa* * | 57.04802 | 45.45 |

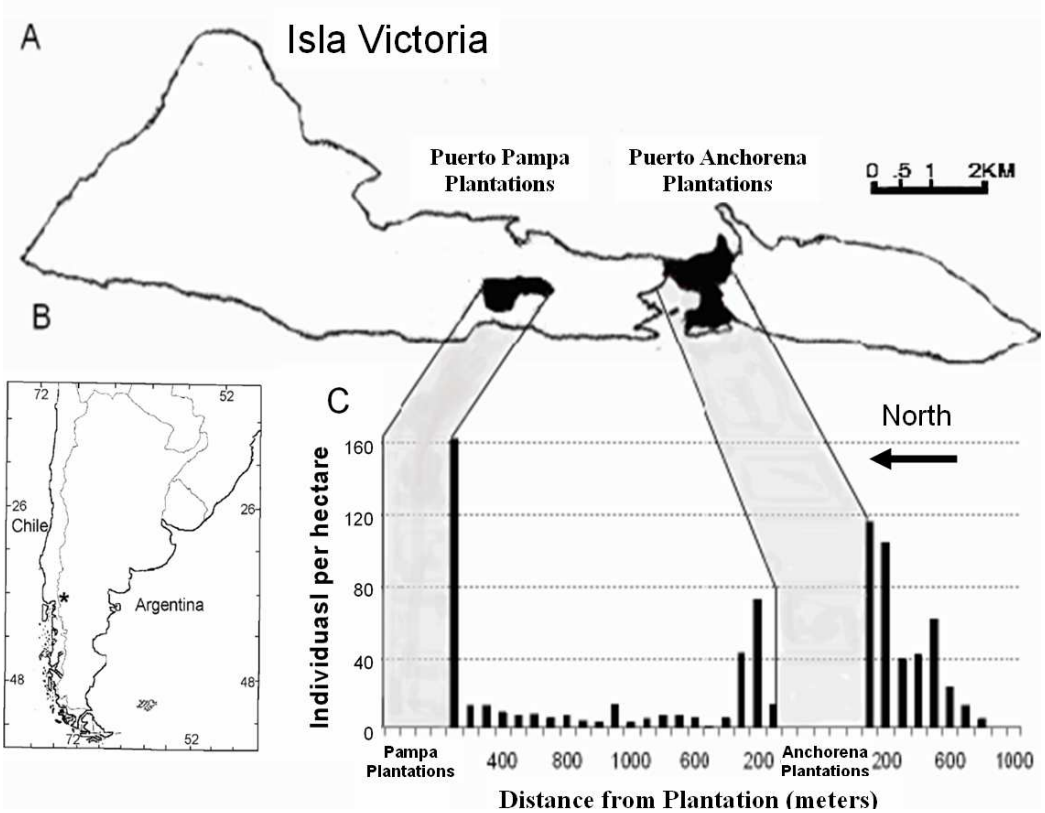


**Figure III-1.** Map of Isla Victoria with abundance of exotic conifers outside plantations.

**a**) Map of Isla Victoria with plantations of exotic Pinaceae in black (Puerto Pampa and Puerto Anchorena plantations). **b**) Location of the study area (black star). **c**) Density of seedlings, saplings, and adults of exotic conifers outside plantations from the Puerto Pampa plantations southward through the Puerto Anchorena plantations, from Simberloff *et al.* (2002).

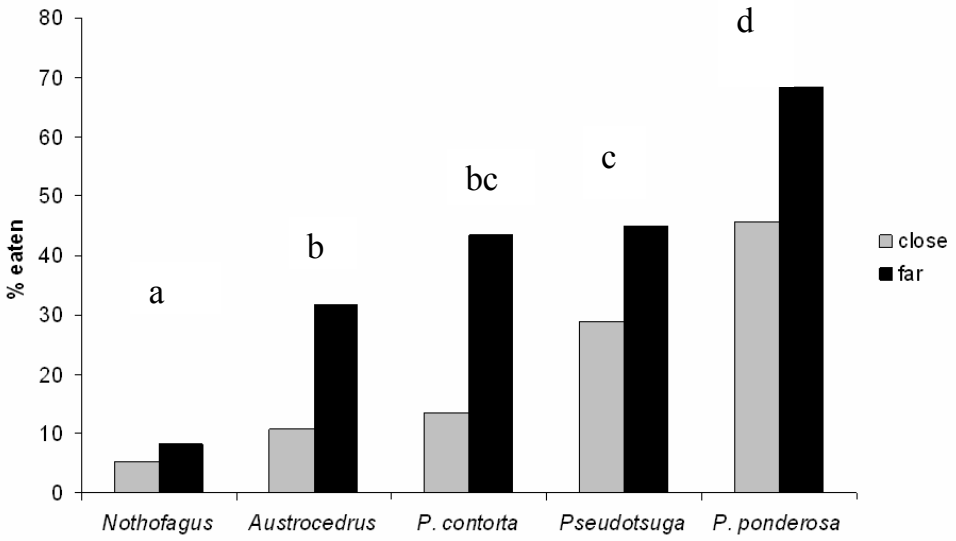


**Figure III-2.** Seeds removed in areas close to and far from plantations after 20-day trials.

Different letters in the graph represent statistically significant differences.

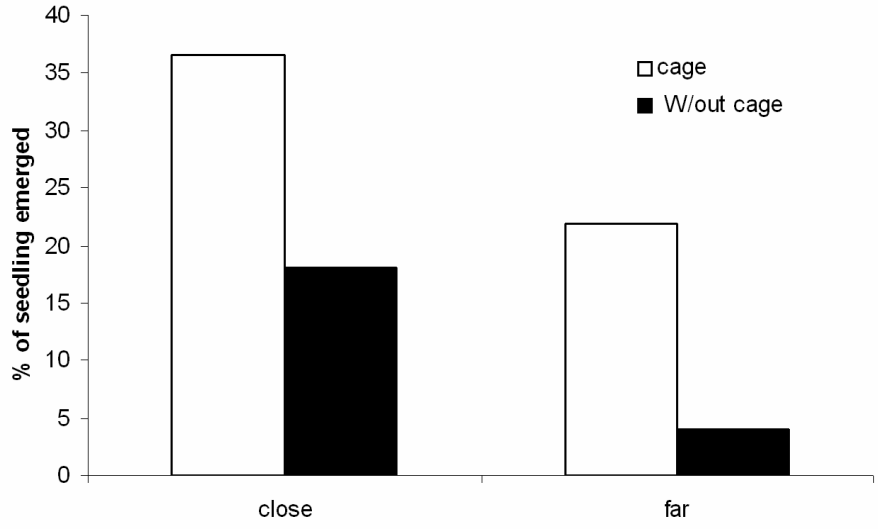


**Figure III-3.** Percent of seedlings emerged among the different treatments for all species pooled.

Seedling emergence rates were statistically different between areas close to and far from plantations and between caged and uncaged treatments (see text for details).

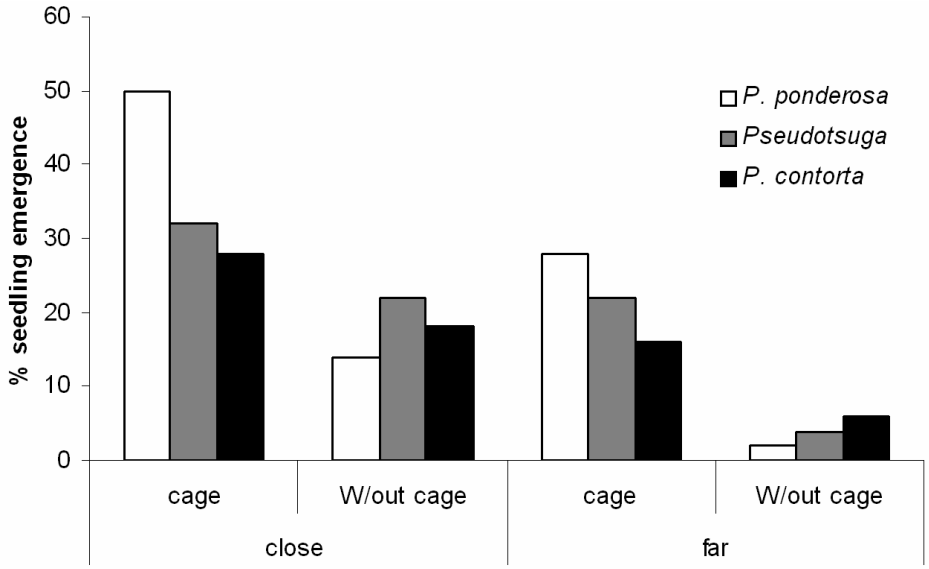


**Figure III-4.** Percent of seedlings emergence for each species in each treatment.

There was a statistical difference between the distance treatments but not among the species. There was also a significant effect of caging (see Fig. III-3).

# CHAPTER IV.

## Lack of soil mutualisms as barrier for Pinaceae invasion

The use of "we" in this part refers to my co-authors and me. I conducted this study in collaboration with Dr. Thomas Horton from SUNY-Syracuse, who was in charge of conducting the molecular techniques aimed to identify the species of mycorrhizal fungi found in this study at his lab, and my advisor Dr. Daniel Simberloff. As the lead author of this article I was responsible for this paper. My primary contributions to this paper included the design of the experiment, statistical analyses and most of the data collection. I also wrote most of the paper.

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**Abstract**

Why particular invasions succeed and others fail is often not well understood. It has recently been suggested that soil biota, mainly pathogens, promote colonization by exotic plants, by reducing negative interactions between such plants and the local soil. Conversely, facilitation has been suggested as an important factor governing biological invasions. For example, mycorrhizal symbiosis or its absence is often claimed to cause success or failure of plant colonization. However, the role of mycorrhizal symbiosis on plant invasion has rarely been explored empirically. We studied the role of ectomycorrhizal (EM) symbiosis on pine invasion on Isla Victoria, Argentina. On this island ca. 80 years ago 135 species of exotic trees, many of them Pinaceae, were introduced, but only a few species have become invasive, and they are found in high densities only near plantations. With a series of field and greenhouse experiments, plus observational and molecular studies, we found that the lack of proper EM fungi could be controlling invasion for at least some of these species. Seedling establishment and growth rates are higher near inoculum sources

(plantations) than far from the inoculum sources. In the greenhouse experiment, the growth of seedlings with soil from areas far from plantations plus fungal inoculum was three times that of seedlings growing with the same soil but without the inoculum. Results of field experiments accorded with those of the greenhouse study. Fungal richness and EM inoculation probabilities were lower for seedlings growing in soil from areas far from plantations. Uninoculated treatments with soil from areas near plantations had four times more colonized seedlings and five times the number of EM fungi species than those with soil from further away. Inoculation increased both the number of species and probabilities of successful fungal colonization. Also, the most abundant species near plantations differ from those common far from plantations, probably because of different dispersal abilities. The lack of proper root mutualisms distant from plantation could be evidence of poor dispersal abilities of species of EM fungi, which may be retarding the invasion. This study shows that positive interactions with soil biota could play a crucial role in plant invasion and colonization and that mycorrhizal interactions could be an important trait explaining plant invasion.

## Introduction

Plant invasion is a major threat to biodiversity conservation and incurs large economic costs. However, why particular invasions succeed and others fail is often not well understood. The role of soil biota, mainly pathogens, has recently been proposed to promote colonization by exotics, by reducing negative interactions between exotic plants and the local soil (Klironomos 2002, Callaway *et al.* 2004). Conversely, facilitation has been suggested as an important factor governing

biological invasions (Simberloff and Von Holle 1999, Richardson *et al.* 2000, Bruno *et al.* 2003). For example, mycorrhizal symbioses or their absence are often claimed to cause success or failure of plant colonization (Horton *et al.* 1999, Klironomos 2002, Callaway *et al.* 2004, Weber *et al.* 2005).

Mycorrhizal fungi can profoundly affect conifer establishment and their absence can therefore limit ability of conifer species to colonize new regions. Pinaceae, for example, depend on ectomycorrhizal (EM) symbiosis to survive, and several plantation forests worldwide have failed for want of adequate EM inocula (Hacskaylo 1972, Mikola 1990, Read 1998). Important characteristics of EM fungi that make their absence a likely factor in failure of conifer invasion are: 1) they are not free-living and 2) they are host-specific to a degree that often prevents native fungi from associating with exotic trees, especially if the tree species introduced is not confamilial with any native species (Molina *et al.* 1992). For example, in New Zealand, exotic Pinaceae do not form symbioses with native fungi associated with *Nothofagus* (Nothofagaceae) (Orlovich and Cairney 2004). In other regions the same phenomenon has been reported, were Pinaceae cannot form symbiosis with native fungi (Mikola 1970, Tedersoo *et al.* 2007).

Another crucial factor that could limit the establishment of exotic Pinaceae is the amount of inoculum in soil. When EM plants are established, the primary inoculation source is thought to be existing hyphal networks associated with plants already present (Selosse *et al.* 2006). Establishment of exotic Pinaceae around plantations is then limited to the root zone of the plantation trees, where seedlings can tap the

preexisting mycelical network. Establishment beyond this root zone is then limited by the presence of spore inoculum, but the mere presence of spores in the soil does not guarantee inoculation, because a high density of spores is needed to colonize conifer roots (Castellano *et al.* 1985, Read 1998). Also, there is evidence of poor dispersal ability of some fungal species outside Pinaceae plantations (Mikola 1953, Lamb 1979, Allen 1987, Allen *et al.* 1992, Davis *et al.* 1996), and some species require animal vectors for long-distance dispersal that may be absent in regions of introduction. These characteristics of Pinaceae and EM fungi make absence of this symbiosis a likely contributor to the lack of Pinaceae invasion in areas where neither the trees nor their associated fungi are native.

Despite the evidence implying an important role for mycorrhizal fungi in conifer invasion, it is frequently suggested that mycorrhizal fungi cannot be limiting invasion owing to their supposed ubiquity (Richardson *et al.* 1994, Reinhart and Callaway 2006). The goal of this research is to test the hypothesis that a paucity of compatible EM fungi on Isla Victoria, Argentina limits invasion by several species of exotic Pinaceae.

## Results

### Greenhouse experiment and field experiment

We conducted a greenhouse and a field experiment in which we planted seeds of three exotic conifers in different soil treatments (see below Methods). We used

*Pseudotsuga menziesii* (Douglas fir), the most abundant non-indigenous tree on the area; the other two species were *Pinus ponderosa* (ponderosa pine) and *P. contorta* (lodgepole pine), which are also invasive elsewhere (Richardson and Rejmánek 2004) but have failed to spread widely on the island, though individual trees have established and flourished. These three species have extensive ranges in their native distribution in temperate regions (Burns and Honkala 1990) and are successfully planted as forestry species in the studied region (Schlichter and Laclau 1998). For the greenhouse experiment we used soils from areas close to plantations (<100 m) where exotic conifers are established, soils from uninvaded areas far from plantations (>1000 m), and soils from each of these two areas plus mycorrhizal inoculum. We also had a control treatment with Isla Victoria sterilized soil. Results differed significantly among species and treatments (Fig. IV-1). We found that inoculum addition increased growth of seedlings by a factor of three in soil from uninvaded areas. Seedlings in sterile soil grew at a rate similar to that of seedlings planted in soil from uninvaded areas that was not inoculated. Field experiments showed results in concordance with the greenhouse study. Establishment probabilities (measured as the proportion of experimental plots with seedlings at the end of the experiment) increased with the addition of EM inoculum, and establishment rates were higher in areas close to plantations (Fig IV-2). Logistic regression showed significant effects of the treatments (higher with inoculum addition vs. sterile inoculum or local soil addition) and location (higher near plantations) on establishment probabilities. For growth we found an effect of location (higher near plantations) but not of inoculum addition. However, seedlings growing far from plantations tended to be bigger if inoculated (Fig. IV-2).

**Mycorrhizal colonization**

We analyzed fungal colonization using morphological and molecular techniques to determine colonization rates, richness, and identities of species. Fungal richness and inoculation probabilities were lower for seedlings growing in soil from areas far from plantations (Tables IV-1, IV-2). Uninoculated treatments with soil from areas near plantations showed four times more colonized seedlings and five times the number of EM fungi species. Inoculation increased both the number of species and probabilities of successful fungal colonization. The most abundant species near plantations differ from those common far from plantations (Fig IV-3). *Wilcoxina mikolae* and *Rhizopogon* sp. dominate seedlings from soil near plantations and were never found far from plantations. This could be because *Wilcoxina* rarely produces fruiting bodies, limiting spore production and spreading mostly by mycelium growth (Trevor *et al.* 2001), while *Rhizopogon* – a hypogeous fungus – needs mammal vectors to dig up the sporocarps and disperse spores in fecal pellets. Far from plantations *Suillus luteus*, a *Pinus*-specific species, was the most abundant species. *Suillus luteus* produces sporocarps prolifically and its fruiting bodies are ubiquitous in the understory of plantations on Isla Victoria as well as in other plantations in the region. The fact that this species produces large numbers of fruiting bodies above ground (epigeous) and therefore wind-dispersed spores could explain its presence far from plantations. Some of the fungal species are *Pinus*- or *Pseudotsuga*- specific species; this specificity could be promoting colonization by species of the one genus but not of the other. In our study, host-specificity could help explain the lack of inoculated *Pseudotsuga menziesii*

growing in soil from areas far from plantations, because *Suillus luteus* does not form EM with *Pseudotsuga menziesii* (Thomas Horton, pers. comm.). However, the presence of some EM fungal species compatible with *P. menziesii* in the area is certain, given that previous observations have shown individuals of this tree species colonized with EM fungi growing far from plantations. Soil properties affect colonization by EM fungi, but we found no soil differences between sites near and far from plantations other than the inoculum potential (Table IV-3). The different species composition and abundance of EM propagules in areas far from plantations diminished chances for pines to find proper EM inoculum.

## Discussion

Our results support the contention that positive interactions are important in biological invasions, as they suggest lack of proper EM fungi impedes Pinaceae invasion on Isla Victoria. The scarcity of EM inoculum lowers the probability of establishment and growth rates of individuals far from the original plantations. These results could help elucidate the lag time sometimes observed in invasion by different tree species elsewhere (Richardson *et al.* 1994) and of tree migration after rapid climate change, such as northern hemisphere colonization of trees at the end of the Pleistocene (Pearson 2006): EM fungal dispersal may require more time than dispersal by plants because these fungi spread by mycelial growth and by building up an efficient spore bank, therefore limiting plant colonization.

Lack of proper mutualisms, such as pollinators or vectors for dispersal, has been shown to be a potential barrier for aboveground invasions (Richardson *et al.* 2000).

We show that this factor can be also important belowground, owing to plant interactions with fungi – one of the least understood groups of invasive organisms (Desprez-Loustau *et al.* 2007). We suggest that facilitation may be a more general mechanism and can help explain patterns of exotic plant invasion, as well as general plant distributional patterns, especially for species associated with mutualists with narrow host ranges.

## Methods

### Greenhouse experiment

To test for the ability of exotic Pinaceae to establish symbioses with compatible EM fungi outside areas influenced by plantations, we conducted a greenhouse experiment with the following soil treatments. **a**) soils from areas near plantations (<100 m) where individuals of the genera *Pinus* and *Pseudotsuga* are established, **b**) soils from uninvaded areas far from plantations (>1000 m), and **c** and **d**) soils from areas close to plantations (**c**) and from uninvaded areas far from plantations (**d**) to which we added soil known to contain EM inoculum (25 $cm^3$, see ). To control for inadvertent EM inoculation in the greenhouse, we used sterilized soil from areas far from plantations. We used 10 pots (1 L each) per species per treatment and added 25 $cm^3$ of soil from plantations known to contain EM inoculum (1/40 of the total volume) to the pots that require inoculum (soil treatments **c** and **d**). To each pot we added five seeds, and only the first seedling to emerge was allowed to grow.

We measured aboveground biomass of seedlings (dry weight of the above ground portion) and analyzed their full root systems under dissecting microscopes to determine presence of EM fungi (based on morphological characteristics) and to catalog EM species by randomly selecting EM root tips for molecular identification (see the mycorrhizal identification section for more detail). We analyzed which fungal species were colonizing the roots of our seedlings using molecular techniques (Gardes and Bruns 1996a, Horton and Bruns 2001).

**Field experiment**

To test the ability of exotic Pinaceae to establish a symbiosis with compatible EM fungi outside plantations, we set up a field experiment. Surface-sterilized seeds of three different species – *Pseudotsuga menziesii*, *Pinus ponderosa,* and *P. contorta* – were planted close to (ca. 100 m) and far from plantations (>1000 m) in late austral winter 2005.

For treatments requiring inoculation, we inoculated seeds with 25 $cm^3$ of soil from Isla Victoria plantations of their own species known to contain EM inoculum, since EM fungi sporocarps are ubiquitous during the fruiting season and thick hyphal mantles are found in them. We planted five seeds per plot per species, in 50 randomly selected locations. Each plot was protected against seed predators with a metal-mesh exclosure (as in chapter III). This experiment has two controls. One consists of sterilized samples (25 $cm^3$) of the soil used to inoculate seeds and the second consists of additions (25 $cm^3$) of soil from the same area where seeds were planted. We followed seedlings for emergence and survivorship monthly for one year and collected

seedlings after one year. We analyzed full seedling root systems as in the greenhouse experiment and measured their aboveground biomass. We analyzed only seedlings established in the growing season (from August to January) because seedlings established later were notably smaller, likely owing to the low rainfall that characterizes summer in this region.

**Mycorrhizal identification**

From the field and the greenhouse experiments, we collected a representative sample of EM root tips from each colonized seedling (five randomly selected EM root tips per seedling). After removal from the seedlings, root tips were stored in 2x CTAB buffer solution (Gardes and Bruns 1996b).

We identified fungal symbionts from ectomycorrhizae by molecular methods – DNA extractions (from single root tips), polymerase chain reaction (PCR) amplifications, and restriction length polymorphism (RFLP) generation – following Gardes and Bruns (1993, 1996a). The ITS region of the nuclear rDNA was amplified using the primers ITS-1f and ITS-4. RFLPs were generated using *Hinf*I and *Dpn*II (New England Biolabs). We compared ITS-RFLP patterns from ectomycorrhizae to each other and to those from voucher specimens of sporocarps collected on Isla Victoria. We also sequenced the nuclear ITS region of one example of each RFLP type. We were unable to obtain ITS sequences from some samples because of mixed extracts or low quality PCR products.

Resulting sequences were subjected to a Blast search in Genbank and UNITE (http://unite.zbi.ee/), a European mycorrhizal database. We identified specimens to genus if the majority of the ITS1 and ITS2 regions overlapped with a sequence in Genbank and had a similarity of 97% or greater (Horton 2002). Sequences with similarities below 97% are identified to family level based on a consensus of the Genbank output. Sequences generated for this study are deposited in Genbank (Table IV.4 [***with the Genbank accession numbers***]).

**Soil analyses**

Because EM colonization can be influenced by edaphic factors (Gehring *et al.* 1998, Erland and Taylor 2002), we characterized soil of the study area. Soil samples collected at our site were analyzed at the Department of Chemistry at the Universidad Nacional del Comahue from samples collected from our study sites. We collected 15 samples from haphazardly selected areas near plantations and 15 areas distant from plantations. Soil was collected at a maximum depth of 15 cm.

For chemical characterization, soils were air-dried and sieved by a 2-mm mesh. The following analyses were conducted: pH in water and 1 M KCl (soil:water/solution 1:2,5); pH in 1 M NaF (1:50); extractable P in 0.5 M $NaHCO_3$ at pH 8.4 (soil solution 1:20) and determined by the ascorbic acid-molybdate method (Blakemore *et al.* 1987, Sparks *et al.* 1996). Subsamples ground to pass a 0.5 mm sieve were analyzed for organic C (Walkley-Black) and total N (semi-micro Kjeldahl) (Sparks *et al.* 1996).

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## Appendix IV:

## Tables and figures

**Table IV-1.** Percent colonization by EM in the studied species, and for all species combined.

Data from field and greenhouse studies. There were significant differences among the treatments without inoculum addition with soils from areas near plantations or far from them ($X^2 = 25.375$, DF = 1, P-value < 0.0001).

| | Near with inoculum addition | Near w/out inoculum addition | Far with inoculum addition | Far w/out inoculum addition |
|---|---|---|---|---|
| *Pinus contorta* | 56 | 65 | 67 | 25 |
| *Pseudotsuga menziesii* | 86 | 88 | 42 | 0 |
| *Pinus ponderosa* | 79 | 62 | 69 | 24 |
| **Average all species** | **73** | **71** | **59** | **16** |

**Table IV-2.** Number of fungal species in each treatment and for all species combined

This is based on RFLF and DNA sequencing analyzes. After sequencing, some species (8 ITS types) did not match any species in Genbank or UNITE databases at the genus or species level and are thus designated as distinctive species. We are confident that these represent unique genotypes because they are not chimeric (same results were obtained if ITS 1 and ITS 2 were blast-searched individually). Further, mycorrhizal fungi are likely underrepresented in DNA databases.

| | Near with inoculum addition | Near with out inoculum addition | Far with inoculum addition | Far with out inoculum addition |
|---|---|---|---|---|
| *Pinus contorta* | 9 | 4 | 1 | 2 |
| *Pseudotsuga menziesii* | 7 | 5 | 3 | 0 |
| *Pinus ponderosa* | 9 | 9 | 4 | 2 |
| **All species combined** | **20** | **16** | **6** | **3** |

**Table IV-3.** Results of soil analyses of areas near and far from plantations.

We found no differences in soil characteristics in the analyzed soils, by 2-tailed t-test (n=15).

| | Near, average (SE) | Far, average (SE) | T-Test (DF=28) | p-value |
|---|---|---|---|---|
| **pH (H2O)** | 6.45 (0.09) | 6.45 (0.11) | 0.03 | 0.97 |
| **pH (NaF)** | 9.01 (0.14) | 8.62 (0.09) | 1.70 | 0.10 |
| **C org (g kg$^{-1}$)** | 78.69 (6.06) | 91.91 (6.44) | -0.96 | 0.34 |
| **N (g kg$^{-1}$)** | 4.87 (0.32) | 5.03 (0.41) | -0.26 | 0.80 |
| **P (µg/g)** | 6.05 (1.70) | 8.26 (1.68) | -0.78 | 0.44 |

**Table IV-4. Sequences obtained in this study, their matched species, and their Genbank accession numbers.**

| Consensus identification | Genbank Accession # |
|---|---|
| *Cadophora finlandica* | EU557316 |
| *Sebacinaceae* sp. 1 | EU557317 |
| Lactarius (deliciosus species group) | EU557318 |
| Ascomycete | EU557319 |
| *Russula drimeia* | EU557320 |
| *Atheliaceae* sp. 1 | EU557321 |
| *Cenococcum geophilum* | EU557322 |
| *Thelephoraceae* sp. 1 | EU557323 |
| *Atheliaceae* sp. 2 | EU557324 |
| *Suillus lakei* | EU557325 |
| Cortinariaceae | EU557326 |
| *Thelephoraceae* sp. 2 | EU557327 |
| *Rhizopogon* | EU562600 |
| *Hebeloma* | EU562602 |
| *Wilcoxina mikolae* | EU562603 |
| *Lactarius* | EU562604 |
| *Thelephoraceae* sp. 3 | EU562605 |
| *Thelephora terrestris* | EU562606 |
| *Suillus luteus* | EU562607 |
| *Sebacinaceae* sp. 2 | EU562608 |
| *Wilcoxina* sp. | EU562609 |

**Figure IV-1.** Aboveground biomass of the species in the greenhouse study under five different treatments.

Treatments from left to right: Soil from areas near plantations plus inoculum, Soil from areas near plantations without inoculum addition, Soil from areas far from plantations plus inoculum, Soil from areas far from plantations without inoculum addition, and sterile soil from Isla Victoria. Grey bars represent samples from soil far from plantations with and without addition of inoculum. There were significant differences among species (ANOVA on log transformed data [due to normality issues] $F_{spp} = 69.21$ DF = 2 P-value < 0.001 ) and treatments (DF = 4 $F_{treatm} = 38.15$ P-value < 0.001), but there was no interaction between species and treatment (DF = 8 $F_{sppxtreatm} = 1.32$ P-value = 0.24). A post-hoc Tukey test grouped the treatments with soil from areas near plantations and the treatment with soil far from plantations with inoculum together, and treatments of soil far away and sterile soil together. All analyses where conducted using SAS 9.13 (SAS institute, Cary, North Carolina, USA).

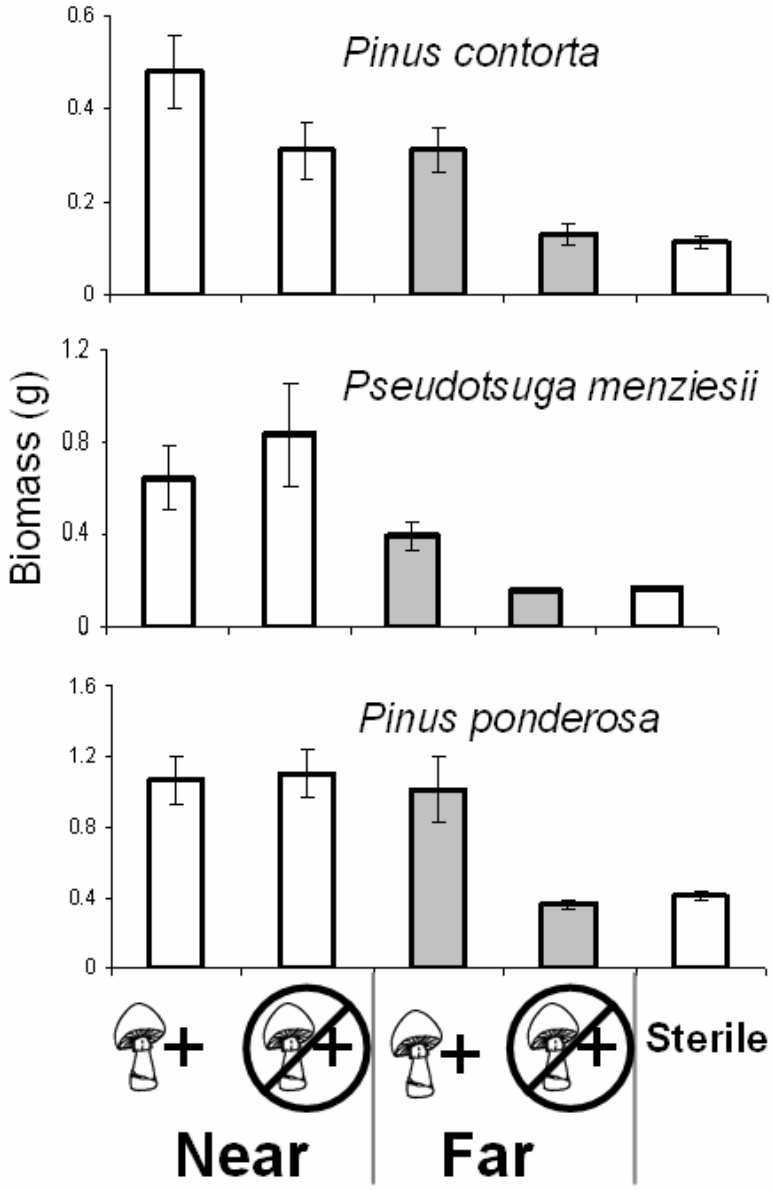
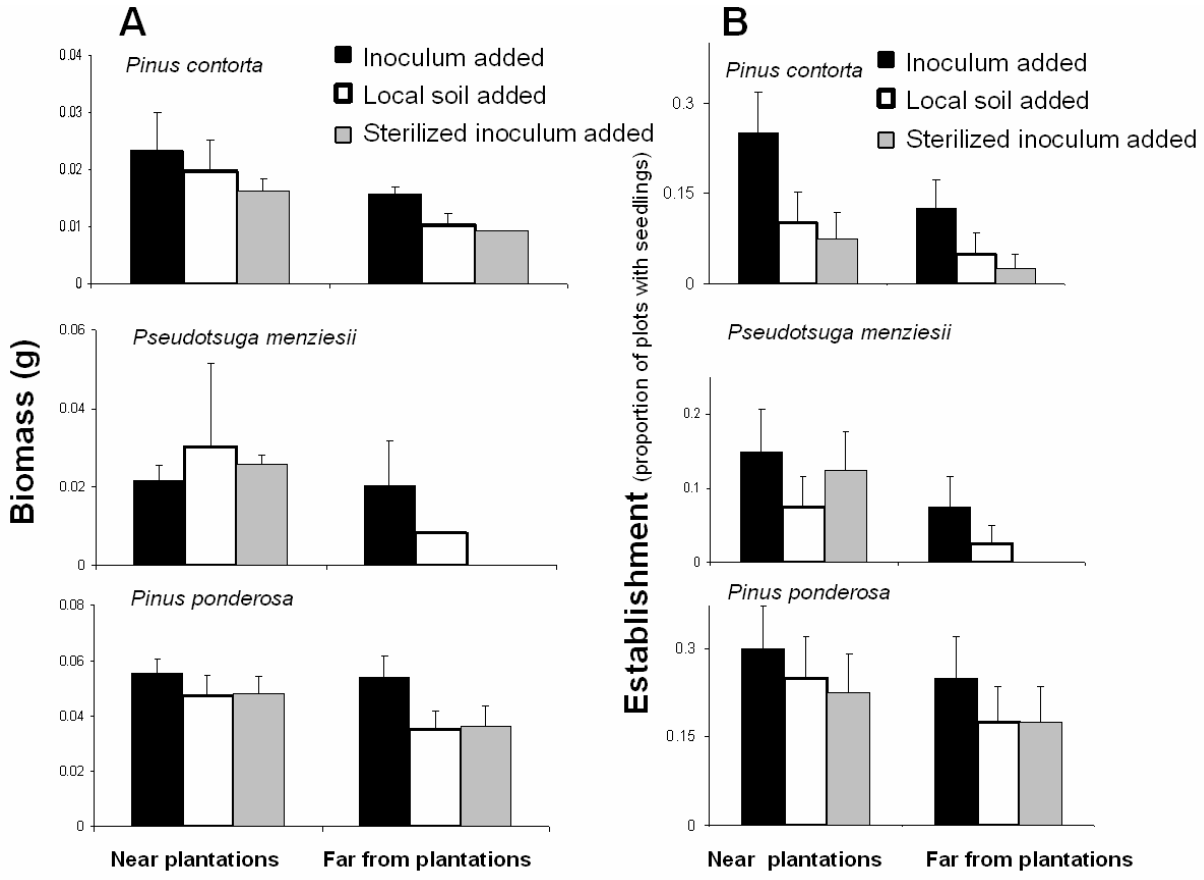

Pinus contorta
Pseudotsuga menziesii
Pinus ponderosa
Biomass (g)
0.6
0.4
0.2
0
1.2
0.8
0.4
0
1.6
1.2
0.8
0.4
0
+
+
+
+
Sterile
Near
Far

**Figure IV-2.** Aboveground biomass and establishment from the field experiments (± SE).

**A)** Aboveground biomass for the three species. We found an effect of location ($F_{area}$= 4.84 P-value = 0.031 Df = 1) on growth but not of EM addition ($F_{treatm}$= 0.99 P-value = 0.376 DF = 2). However there was a trend for seedlings growing far from plantations to be bigger if they had ECM inoculum. **B)** Proportion of plots with seedlings established at the end of the experiment. Analysis using logistic regression showed that there was a significant difference in treatment effect (DF = 2 $X^2_{treatm}$ = 10.31 P-value < 0.006), with plots with the EM addition treatment having more seedlings than the other two treatments. There were also differences in effect of species (DF = 2, $X^2_{spp}$ = 32.8 P-value < 0.001) and distance (DF = 1 $X^2_{area}$= 4.9 P-value = 0.026) on establishment probabilities.


A
B
Inoculum added
Local soil added
Sterilized inoculum added
Pinus contorta
Pseudotsuga menziesii
Pinus ponderosa
Biomass (g)
Establishment (proportion of plots with seedlings)
Near plantations
Far from plantations

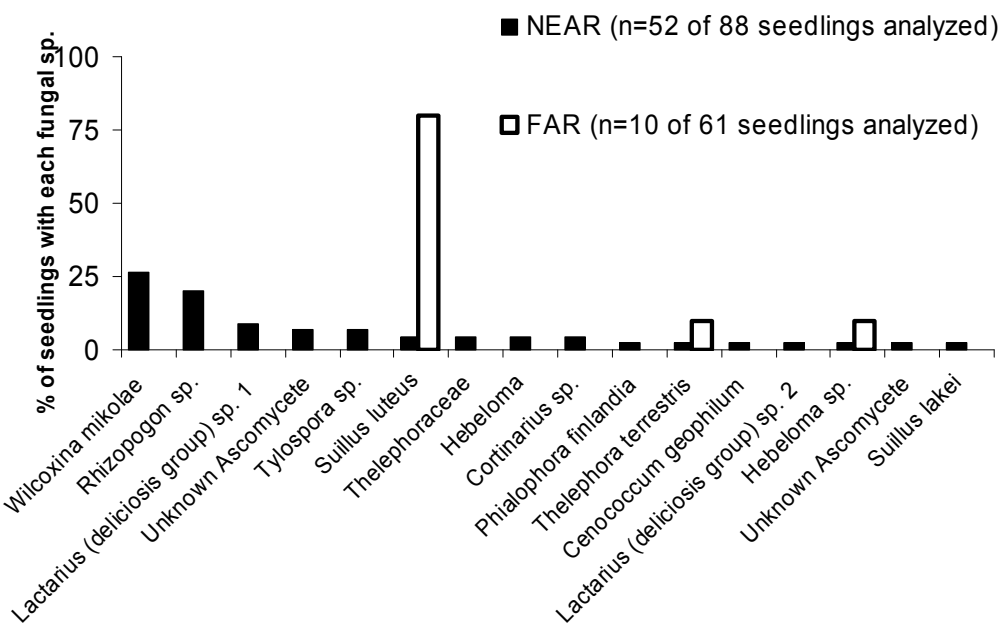



**Figure IV-3.** Percent of mycorrhizal species colonizing roots of seedlings from field and greenhouse experiment combined**.**

This figure does not include seedlings from the experiments with inoculum addition. The five most abundant species near plantations were absent far from plantations. The total number of species found near plantations was 16, and far from plantations was 3. Some species of the genus *Rhizopogon* are specific symbionts of the genus *Pinus* and others are specific symbionts of *Pseudotsuga*. *Suillus luteus* and the *Lactarius deliciosis* group are EM fungi only with *Pinus* species.

## Vita

Martin Nuñez was born in Neuquen, Argentina on October 22nd 1977. He attended public school from 1984 and 1985 in Alicura, Neuquen and from 1986 in Bariloche, Rio Negro, where he graduated from the E.N.E.T N°1 Jorge Newbery high school with the degree of Technician in Electricity and Mechanics in 1996. In February 1997 he entered the Universidad del Comahue, where in October 2002 he received the degree of *Licenciado en Ciencias Biologicas*. In August 2003 he started the Ph.D. program in Ecology and Evolutionary Biology at the University of Tennessee, Knoxville. The doctoral degree was awarded in May 2008.